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# ANDROGEN EFFECTS ON FOOD INTAKE, BODY WEIGHT AND CARCASS COMPOSITION IN MALE RATS

A Dissertation Presented

By

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ABSTRACT

Androgen Effects on Food Intake, Body Weight and Carcass Composition in Male Rats

(September, 1981)

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Long-term treatment with low doses (0.2 mg/day) of the aromatizable androgen, testosterone propionate (TP), increased food intake and body weight gain in gonadectomized (GDX), adult male rats. Dietary self-selection studies revealed that the increased intake was due to enhanced consumption of all dietary components. Prolonged treatment with higher doses of TP (2.0 mg/day) reduced weight gain compared to the lower dose of TP, and increased protein, but not carbohydrate intake, and reduced carcass fat. The elevated protein intakes of both TP-treated groups were associated with increased carcass protein contents.

The nonaromatizable androgen, 5α-dihydrotestosterone propionate (DHTP; 0.2 or 2.0 mg/day), also increased protein (but not carbohydrate) intake and weight gain in GDX male rats, but it did not alter carcass composition. Unlike TP, both doses of DHTP were equally effective, but neither dose of DHTP was as

effective as the low dose of TP in stimulating protein and carbohydrate intake and body weight gain.

The results of these experiments suggest: 1) Androgens can increase selection of dietary protein whether or not they exert significant protein anabolic effects. 2) The 5α-reduced metabolite of testosterone, 5α-dihydrotestosterone, is not the major metabolite responsible for the increases in protein and caloric intake and in body weight caused by TP. 3) The decreases in carbohydrate intake and adiposity in rats given the high dose of TP may be mediated by aromatized (estrogenic) metabolites of the androgen.

Further support for the hypothesis that high doses of TP decrease weight gain and adiposity via estrogenic metabolites is provided by the finding that concurrent treatment with androsta-1,4,6-triene-3,17-dione (ATD), which blocks aromatization of androgens to estrogens, prevented the weight-reducing effects of high doses of TP. Furthermore, prolonged treatment with 1.0 mg TP/day also depleted cytoplasmic estrogen receptors and reduced lipoprotein lipase (LPL) activity in epididymal adipose tissue in GDX males (similar changes follow estrogen treatment in female rats). Both effects were blocked by concurrent treatment with ATD. These results suggest that estrogenic androgen metabolites may reduce weight

by direct actions on adipose tissue metabolism.

Copulation has been shown to increase testosterone levels in male rats. Mated male rats decreased body weight, but this decrease was not associated with changes in caloric intake or intake of specific nutrients; nor was LPL activity affected by sexual activity. Thus, copulation-induced reductions in weight may be mediated by mechanisms other than those cited above for rats receiving high doses of TP.

In addition to peripheral effects, testosterone or its estrogenic metabolites may also act on central sites to influence eating. Implants of TP in the ventromedial hypothalamus (VMH) reduced food intake in GDX males. Implants of TP in the anterior hypothalamus-preoptic area or VMH implants of DHTP did not alter food intake.

Finally, a single injection of estradiol has been shown to decrease food intake in castrated rats fed a high fat diet, but this treatment has no effect on intake in GDX males fed a chow diet. It was hypothesized that if high doses of TP affect food intake, in part, via estrogenic metabolites, then GDX male rats fed a high fat diet and given high doses of TP should eat less than similarly treated rats fed chow. No evidence was found

to support this hypothesis. Rats in both diet groups responded similarly to TP treatment.

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# CHAPTER I

## INTRODUCTION

In many mammalian species, including man, the male of the species is heavier than the female (Slob and van der Werff ten Bosch, 1975; Tanner, 1962), although exceptions do occur (e.g., female hyenas, hamsters and Saguinus New World monkeys are larger than their male counterparts; Hoyenga and Hoyenga, 1979). Adult male rats are longer and heavier, and eat more than do similarly aged females (King, 1915; Wang, 1924). The sex differences in food intake and body weight in rats can be attributed largely to the influences of gonadal hormones (for reviews, see Wade, 1975; 1976; Wade and Gray, 1979).

### Mechanism of Action of Steroids

All steroid hormones are believed to have a common mode of action (for reviews, see Gorski and Gannon, 1976; Mainwaring, 1977). Steroids are transported in the blood in the form of stable complexes with plasma proteins. The hormones enter all cells, purportedly by the process of passive diffusion (although specific transport mechanisms have been proposed; Mainwaring, 1977). Within target cells (such as those in uterus and hypothalamus)

the steroids bind with steroid specificity and high affinity to cytoplasmic receptor proteins. Changes in the configurations of the hormone-receptor complexes occur, resulting in activated complexes. These activated hormone-receptor complexes are translocated into cell nuclei where they are retained at sites in chromatin, causing changes in RNA synthesis which result in altered protein synthesis.

Many steroids exert their effects without chemical modification. However, some hormones, primarily the androgens, are subject to extensive metabolism within target cells prior to binding to cytoplasmic receptors. For example, testosterone is rapidly metabolized by 5α-reductase to 5α-dihydrotestosterone (DHT) in many peripheral tissues (e.g., prostate, seminal vesicles and epididymis); and it is DHT that binds to the androgen receptor and subsequently produces the tissue-specific response (Liang, Tymoczko, Chan, Hung and Liao, 1977). Testosterone and certain other steroids of the 19-carbon series can also be converted (aromatized) to estrogens by the aromatase enzyme system in some peripheral tissues (Mainwaring, 1977). In the central nervous system, enzymes associated with both 5α-reduction and aromatization have been identified, and they are primarily concentrated in the hypothalamus and amygdala (Celotti,

Massa and Martini, 1979; Goy and McEwen, 1980).

Historically, the growth-promoting influences of hormones such as DHT on the male reproductive tract have been termed androgenic effects. Testosterone and some of its metabolites also stimulate growth in non-reproductive tissues such as skeletal muscle, kidney and liver. The effects of androgens on these tissues are associated with decreased urinary nitrogen excretion (indicating enhanced protein synthesis), and are referred to as anabolic actions.

## Gonadal Hormone Effects on Food Intake and Body Weight

The practice of ablative surgery on the male reproductive glands appears to date back to earliest recorded history. The first scientific investigation of the effects of castration may have been performed by Aristotle, who recorded the effects of removal of the gonads in the cockerel and compared them with changes observed in castrated men (Mainwaring, 1977; Turner and Bagnara, 1971). Although orchiectomy is an ancient practice, surprisingly little is known about the effects of this procedure on body weight and food intake. Admittedly, purified testicular hormones were not readily available for use in hormone replacement studies prior to the isolation of testosterone by David and colleagues

in 1935 (although there is evidence that alchemists of ancient Cathay isolated and purified steroid hormones; Mainwaring, 1977). In the mid 1930's to the mid 1940's, Kochakian used fat-soluble extracts from male urine to investigate the effects of androgens on nitrogen metabolism and body weight in castrated dogs. He later extended his studies to include the effects of testosterone on nitrogen balance in rats (Kochakian, 1975). Yet it was not until 1976 that the effects of two androgens on food intake in rats were unequivocally established (Gentry and Wade, 1976a).

In contrast, a comparatively vast amount of information is available on the effects of ovariectomy and ovarian hormone replacement on food intake, body weight and adiposity in female rats (Wade, 1975; 1976; Wade and Gray, 1979). Because some of the effects of particular testicular hormones on the above parameters appear to be mediated by estrogenic metabolites of these steroids, the influence of ovarian hormones on eating and body weight regulation in female rats will be reviewed briefly.

Female rats. The ovarian hormones, 17β-estradiol and progesterone, have marked effects on food intake and body weight in adult female rats. Withdrawal of these hormones by ovariectomy results in increased food intake and body

weight gain. Beginning approximately 30 days after ovariectomy, food intake returns to the level of intact rats, and body weight stabilizes at a level 25% above that of control rats (Gentry and Wade, 1976a; Roy and Wade, 1977; Stotsenburg, 1913; Tarttelin and Gorski, 1973). The change in weight observed after ovariectomy is the result of increases in all carcass components. The most marked increase, however, occurs in carcass fat content which is 1.6 to 2 times greater (depending on diet) in ovariectomized (OVX) rats than it is in intact female rats (Leshner and Collier, 1973).

The effects of ovariectomy on food intake and body weight are reversed by 1) systemic injections of estradiol benzoate (EB; Mook, Kenney, Roberts, Nussbaum and Rodier, 1972; Roy and Wade, 1977; Wade, 1975), or 2) estradiol delivered directly into the ventromedial hypothalamus (VMH) in OVX rats (Jankowiak and Stern, 1974; Wade and Zucker, 1970). When OVX rats are given estradiol body fat levels return to those of intact control rats (Salans, 1971; Gray and Wade, 1981).

In the absence of estrogen, progesterone has no influence on food intake, body weight or carcass composition in OVX rats (Galletti and Klopper, 1964; Hervey and Hervey, 1966; Ross and Zucker, 1974). However, when progesterone is given concurrently with estradiol,

adiposity increases and transient increases in eating and body weight gain take place relative to rats treated with estradiol alone (Galletti and Klopper, 1964; Hervey and Hervey, 1966; 1967; 1968; Wade, 1975). Similar changes are seen following progesterone treatment in intact rats (Hervey and Hervey, 1966; Wade, 1975).

That the effects of the hormonal manipulations reported above are not merely the result of pharmacological doses of gonadal hormones is indicated by the fact that food intake, body weight and adiposity vary predictably with changes in endogenous hormone levels. Food intake and body weight decrease when circulating estradiol levels are high (proestrus; Hori, Ide and Miyake, 1968; ter Haar, 1972; Yoshinaga, Hawkins and Stocker, 1969), and increase when estradiol titers decrease (diestrus; Brobeck, Wheatland and Strominger, 1947; Rothchild, 1967). In addition, during pregnancy or pseudopregnancy, when progesterone levels are high, food intake, body weight and adiposity increase in rats (Hashimoto, Hendricks, Anderson and Melamey, 1968; Knopp, Saudek, Arky and O'Sullivan, 1973; Wade and Zucker, 1969).

Ovarian hormones may act at several sites to cause changes in food intake and body weight. For years it had been assumed that gonadal hormones influenced

body weight by acting on specific brain sites containing specific estrogen and progestin receptors to modulate eating behavior, thus influencing body weight (Wade, 1976). As discussed by Wade and Gray (1979) several lines of evidence suggest that this view of hormone action is incomplete. Implants of EB in the VMH (but not in other diencephalic sites) do cause reductions in eating and weight gain in female rats (Jankowiak and Stern, 1974; Wade and Zucker, 1970). However, since ovariectomy increases and EB treatment decreases body weight and food intake in rats with large hypothalamic lesions (Beatty, O'Briant and Vilberg, 1975; King and Cox, 1973), the hypothalamus cannot be the only site of action. In addition, changes in food intake that follow treatment with steroid hormones cannot alone account for the observed changes in body weight. When progesterone-induced hyperphagia is prevented in intact rats by restricting food intake to pretreatment levels, mean body weight still increases significantly compared to controls (Hervey and Hervey, 1968). Similarly, OVX rats must be restricted to approximately 80% of their pre-OVX ad libitum food intake to prevent the rise in body weight that follows ovariectomy (Roy and Wade, 1977). Furthermore, when OVX rats are pair fed with EB-treated OVX rats, they do not

lose weight. Thus, hyperphagia is not necessary for progesterone- or ovariectomy-induced weight gain, nor is hypophagia sufficient to produce decreased weight following estrogen treatment in OVX rats.

These studies suggest that ovarian hormones do not alter body weight by acting solely on the brain to alter ingestive behaviors. In 1979, Wade and Gray proposed that ovarian steroids might also act directly on peripheral tissues, such as those involved in triglyceride storage, to influence body weight, food intake and adiposity. For example, in adipose tissues, they suggested that ovarian hormones might act via high-affinity steroid-specific cytoplasmic estrogen (Wade and Gray, 1978) and progestin receptors (Gray and Wade, 1979) to alter activity of the enzyme lipoprotein lipase (LPL) in female rats. This enzyme plays an essential role in hydrolysis of plasma triglycerides and their subsequent storage in adipose tissues (Scow, Hamosh, Blanchette-Mackie and Evans, 1972). Thus, changes in adipose tissue LPL activity alter the availability of circulating triglycerides for storage. Estradiol treatment decreases adipose tissue LPL activity in OVX rats (Gray and Wade, 1980; Hamosh and Hamosh, 1975) causing decreased fat storage. Progesterone treatment of intact female rats or of OVX rats primed with estrogen increases LPL

activity (Gray and Wade, 1980; 1981; Kim and Kalkoff, 1975) and adiposity (Galletti and Klopper, 1964; Gray and Wade, 1981).

Ovarian hormones also act on other tissues and enzyme systems to modulate triglyceride clearance and synthesis. For example, rat liver contains cytoplasmic estrogen receptors (Eisenfeld and Aten, 1979), and estrogens increase hepatic triglyceride synthesis (Kim and Kalkoff, 1975; 1978) and modulate production of apoproteins which serve as enzyme cofactors for LPL and structural elements of plasma lipoproteins (Kim and Kalkoff, 1978; Patsch, Kim, Wiest and Schonfeld, 1980).

Wade and Gray suggested that changes in food intake that follow treatment with ovarian steroid might be a consequence of altered levels of circulating fuels. For example, the decreased food intake of estradiol-treated rats might, in part, be due to increased availability of plasma triglycerides. Changes in body weight and composition might result from direct metabolic responses to ovarian hormones. While there is much support for this latter suggestion, recent data indicate that hypertriglyceridemia is not necessary for the estradiol-induced decrease in food intake (Gray and Wade, 1981; Ramirez, 1980; 1981). Thus, other mechanisms may also be involved in modulating the effects of estrogen

on food intake.

Male rats. Castration of adult male rats results in a decreased rate of weight gain and a reduction in food intake (Gentry and Wade, 1976a; Kakolewski, Cox and Valenstein, 1968). The decrease in consummatory behavior generally does not appear until approximately one month post-castration. The attenuation in body weight is apparent immediately after orchiectomy and is mostly due to tissue loss. Over time, however, body weight gain slows such that by three months post-castration, gonadectomized (GDX) males weigh 8% less than intact control rats (Gentry and Wade, 1976a). The delay between removal of testicular hormones and changes in androgen-dependent processes is not unprecedented, a similar lag period is seen for the reduction of sexual responsiveness after castration (Bloch and Davidson, 1968; Stone, 1927).

When castrated male rats are given injections of testosterone propionate (TP) or 5α-dihydrotestosterone propionate (DHTP), food intake and body weight increase (Gentry and Wade, 1976a). Unlike the effects of DHTP, the effects of testosterone replacement on ingestive behavior and weight gain are dose-dependent. Low doses of TP ($\leq$ 200 µg/day) increase food intake and body weight gain (Gentry and Wade, 1976a; Rubinstein and Solomon, 1940). Higher doses of TP (1-2 mg/day) also produce

increases in these parameters, but within one to two weeks, body weight and food intake decline (Gentry and Wade, 1976a). The decrease in body weight that follows prolonged treatment with high concentrations of TP was termed "the wearing-off effect" (Kochakian, 1975), since it was believed that the effects of testosterone simply wore off over time. However, the weight changes following administration of high doses of this androgen are primarily due to decreases in adiposity (Hervey and Hutchinson, 1972; Kochakian, 1975), suggesting that these decreases may in fact be mediated by estrogenic products of TP. Further evidence for this hypothesis is provided in later chapters.

The studies that follow were designed to further explore the influence of gonadal hormones on food intake, body weight and carcass composition in male rats. Some of the sites at which steroid hormones act and mechanisms by which they act to induce changes in these variables were investigated.

# CHAPTER II

# TESTOSTERONE EFFECTS ON FOOD INTAKE AND BODY WEIGHT IN MALE RATS: ROLE OF ESTROGENIC METABOLITES

## Experiment I: Effects of TP on Food Intake and Body Weight in GDX Rats Treated with an Aromatization Inhibitor

It has been postulated that the reduction in weight gain seen after treatment with high doses (1-2 mg/day) of TP is due, in part, to estrogenic metabolites of TP (Gentry and Wade, 1976a; Hervey and Hutchinson, 1972). That DHTP, an androgen that cannot be aromatized to estrogens, does not reduce food intake or body weight gain even when administered in high doses (20 mg/day; Gentry and Wade, 1976a) lends credence to this hypothesis. In addition, concurrent treatment with progesterone blocks both estradiol-induced decreases in body weight (Wade, 1976) and the weight-reducing effects of high doses of TP (Gentry and Wade, 1976a).

Experiment I was designed to test the aromatization hypothesis. Castrated male rats bearing silastic capsules containing the aromatase inhibitor, androsta-1,4,6-triene-3,17-dione (ATD), were given daily injections of a high dose of TP, and food intake and body weight were measured. ATD has been shown to prevent estrogen production from testosterone in several tissues (Brodie,

Schwarzel and Brodie, 1976; Lieberburg, Wallach and McEwen, 1977; Schwarzel, Kruggel and Brodie, 1977) and blocks the effects of aromatizable androgens on male and female sexual behavior (Christensen and Clemens, 1975; Gladue, Dohanich and Clemens, 1978; Morali, Larsson and Beyer, 1977), and on sexual differentiation (Gladue and Clemens, 1980; McEwen, Lieberburg, Chaptal and Krey, 1977) in rats. If indeed estrogenic metabolites are responsible for some of the changes in body weight and food intake that follow treatment with high doses of TP, then ATD should prevent or attenuate these changes in rats receiving concurrent treatment with high doses of TP.

Method.

Animals and housing. Forty-five male CD-strain rats (body weight 250-300 g at the time of surgery) obtained from Charles River Breeding Laboratories were used in the experiment. The animals were individually housed in Wahmann LC-75 wire bottom cages and were given free access to food pellets (Purina laboratory chow) and tap water. A 12-hour-light: 12-hour-dark cycle (lights on at 0800 hr) was maintained throughout the experiment.

Steroids and silastic capsules. TP and cholesterol (C) were purchased from Sigma Chemical Co., and ATD was purchased from Steraloids Inc. Implants of ATD

were each made from 10 cm Silastic tubing (1.47 mm inner diameter; 1.96 mm outer diameter; medium grade tubing; Dow Corning) and contained approximately 100 mg of ATD. The capsules were sealed at both ends with wood plugs and Silastic adhesive, cleaned with ethanol, and allowed to cure at room temperature for 24 hours. Prior to implantation, the capsules were incubated for 28 hours following the procedure described by Smith, Damassa and Davidson (1977). Capsules of C (3 cm) were prepared in similar fashion and implanted in all animals not receiving ATD.

Procedure. All animals were castrated under methoxyflurane (Metofane; Pittman-Moore) anesthesia through a single scrotal incision. Beginning 4 weeks after surgery all animals received daily subcutaneous injections of 0.1 ml sesame oil. Food intake including spillage (to the nearest 0.1 g) and body weight (to the nearest g) were measured every third day at approximately 1300 hr throughout the experiment. After 9 days of oil injections, animals were divided into five hormone treatment groups matched for body weight and food intake (Table 1), and rats were given a subcutaneous implant of C or ATD under methoxyflurane anesthesia. The groups (n=9 each) were: C + Oil: C implant and 0.1 ml oil/day; ATD + Oil: ATD implant and 0.1 ml

oil/day; C + 1 mg TP: C implant and 1 mg TP/day: ATD + 1 mg TP: ATD implant and 1 mg TP/day; C + 0.2 mg TP: C implant and 0.2 mg TP/day. All doses of TP were dissolved in 0.1 ml of sesame oil.

Data analysis. Data for the three days prior to hormone treatment were used as baseline. Body weight data were converted to % baseline for statistical analysis. Food intake data were converted to calories (utilizable calories in chow diet = 3.61 kcal/g; Corbit and Stellar, 1964). Comparisons across groups were made using one-way analyses of variance. Significant $F$ values ($p<.05$) were followed by Newman-Keuls post hoc tests.

Results.

Body weight (Figure 1, Table 1). Baseline body weights did not differ significantly across groups. After 15 days of treatment, the three groups receiving TP injections (C + 1 mg TP, ATD + 1 mg TP, C + .2 mg TP) had gained significantly more weight than their respective oil control groups, $F$ (4,40) = 14.67, $p<.00001$ ($p< .01$ all post hoc tests). Weight gains of the three TP groups did not differ significantly from one another, and no significant differences were found between the two oil-treated groups during the first half of the experiment. The thirty days of treatment significantly affected body

weight gain, $F$ (4,40) = 11.88, $p<.00001$. At the end of the experimental period the .2 mg TP + C and 1 mg TP + ATD groups had gained significantly more weight than their respective oil control groups ($p<.01$, both tests). In addition, both the .2 mg TP + C and 1 mg TP + ATD groups gained significantly more weight than the 1 mg TP + C group ($p<.01$, $p<.05$, respectively). No significant differences were found between the two oil groups.

Food intake (Figure 1, Table 1). No significant differences were found in baseline food intakes across groups. In contrast to the findings for body weight, during the first 15 treatment days only the group receiving .2 mg TP + C ate significantly more than its respective oil control group, $F$ (4,40) = 3.95, $p<.01$ ($p<.01$, Newman-Keuls). Neither the 1 mg TP + C and 1 mg TP + ATD nor the Oil + C and Oil + ATD groups differed significantly from each other. During the last 15 days of treatment, all three TP treatment groups ate significantly more than their respective oil control groups, $F$ (4,40) = 7.09, $p<.0002$ ($p<.05$ or better all post hoc comparisons). The three treatment groups did not differ significantly from one another during this period, nor was food intake significantly different for the Oil + C group compared to the Oil + ATD group.

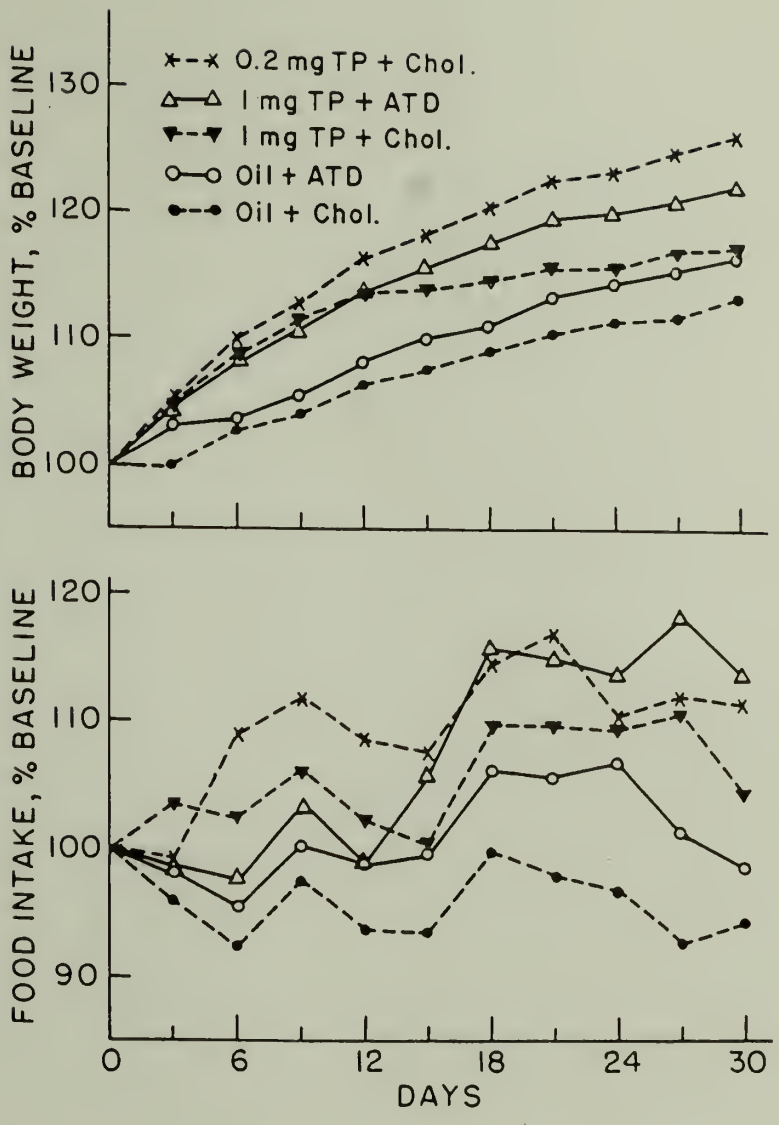



Fig. 1. Effect of TP in GDX rats bearing Silastic implants containing cholesterol or androsta-1,4,6-triene-3,17-dione on body weight and food intake.

TABLE 1

EFFECTS OF TP ON BODY WEIGHT AND FOOD INTAKE IN ATD- OR CHOLESTEROL-TREATED RATS

| GROUP | BODY WEIGHT (g) | | | FOOD INTAKE (kcal/day) | | |
|---|---|---|---|---|---|---|
| | | DAY | | | DAYS | |
| | BASELINE | 15 | 30 | BASELINE | 1-15 | 16-30 |
| OIL + C | 352.3 ±15.4 | 378.8 ±17.3 | 399.0 ±17.4 | 98.4 ±3.8 | 90.4 ±3.2 | 91.9 ±2.7 |
| OIL + ATD | 354.1 ± 9.6 | 385.0 ±12.2 | 408.4 ±12.2 | 98.4 ±1.9 | 92.6 ±2.8 | 97.5 ±2.5 |
| 1 mg TP + C | 352.4 ± 8.6 | 401.2 ±10.6 | 413.2 ± 9.6 | 99.0 ±3.4 | 98.8 ±2.4 | 104.2 ±2.5 |
| 1 mg TP + ATD | 351.6 ±10.9 | 407.2 ±14.4 | 431.4 ±17.1 | 99.9 ±3.6 | 97.4 ±3.5 | 111.6 ±4.3 |
| .2 mg TP + C | 350.4 ±10.9 | 413.3 ±12.6 | 442.8 ±14.2 | 102.7 ±3.0 | 105.9 ±3.2 | 111.6 ±3.8 |

Data are presented as mean ± S.E.M.

## Experiment II: Effects of TP on Cytoplasmic Estrogen Binding and LPL Activity in Epididymal Adipose Tissues of GDX Rats Treated with an Aromatization Inhibitor

Earlier findings indicate that long-term treatment with high doses of TP decreases the amount of carcass fat in male rats (Hervey and Hutchinson, 1972; Kochakian and Webster, 1958). It has been shown that estrogens may act directly on adipose tissues (Wade and Gray, 1978), and that aromatization of androgens occurs in some adipose tissues (Kley, Deselaers, Peerenboom and Kruskemper, 1980;

Nimrod and Ryan, 1975; Perel and Killinger, 1979). Thus, estrogenic products of testosterone might possibly be acting on adipose tissues to alter carcass composition. Estradiol may, in part, decrease carcass fat content by decreasing adipose tissue LPL activity. Recent studies have demonstrated that male and female rat adipose tissues contain high-affinity, steroid-specific cytoplasmic estrogen receptors (Gray and Wade, 1980; Wade and Gray, 1978) and that estrogen treatment lowers adipose tissue LPL activity in rats of either sex (Hamosh and Hamosh, 1975; Wilson, Flowers, Carlile and Udall, 1976).

In Experiment II the effects of long-term TP treatment, with or without supplemental ATD, on cytoplasmic receptor concentration and LPL activity in epididymal adipose tissue were examined in GDX male rats. In addition, retroperitoneal and epididymal adipose tissue weights were recorded. These two fat pads were chosen because they have been shown to contain saturable, steroid-specific cytoplasmic estrogen receptors. If decreased carcass fat content of males given high doses of TP is mediated by estrogenic metabolites, then this hormone treatment should both deplete cytoplasmic estrogen receptors (estrogen receptors should be translocated to cell nuclei) and reduce LPL activity in rat

adipose tissues. These effects should be blocked or attenuated in rats receiving ATD.

Method.

Animals and housing. Rats were those used in Experiment I.

Procedure. At the completion of the injection period (Experiment I) all rats were killed by decapitation. Epididymal and retroperitoneal adipose tissues were dissected and weighed. Because epididymal fat pads have the highest concentration of cytoplasmic estrogen receptors in male rat adipose tissues (Gray and Wade, 1980), epididymal fat pads were used for cytoplasmic estrogen receptor studies (left pad) and LPL assay (right pad).

Cytoplasmic estrogen receptor assay. Left epididymal fat pads were homogenized in ground glass tissue grinders (Duall size 23; Kontes) at a concentration of 400 mg/ml buffer containing 10 mM Tris-HCL, 1.5 mM EDTA, 12 mM monothioglycerol and 10% (v/v) glycerol, pH 7.4 (TEMG). Cytoplasmic estrogen binding was measured as described by Wade and Gray (1978) with the exception that TEMG buffer was used in this assay. Protein concentrations were measured according to the method of Lowry, Rosebrough, Farr and Randall (1951).

Lipoprotein lipase assay. Right epididymal fat

pads were homogenized at a concentration of 250 mg tissue per ml of medium containing 0.25 M sucrose and 1 mM EDTA, pH 7.4. Postmitochondrial supernatants were obtained following centrifugation of the homogenate at 12,000 g for 15 minutes. Lipoprotein lipase activity was assayed by the method of Schotz, Garfinkel, Huebotter and Stewart (1970).

Data analysis. Data were analyzed using one-way analyses of variance. Significant $F$ values ($p<.05$) were followed by Newman-Keuls post hoc tests.

Results. No significant differences were found in epididymal or retroperitoneal fat pad weights across groups. However, the ATD + 1 mg TP group had a mean epididymal fat pad weight that was at least 50% higher than those of the other groups. In addition, retroperitoneal fat pads from the C + 1 mg TP group (mean fat pad weight $\pm$ S.E.M. = 3.05 $\pm$ 0.25 g) had lower weights than those from the .2 mg TP + C (4.40 $\pm$ 0.26 g) and the oil control groups (4.40 $\pm$ 0.48 and 4.82 $\pm$ 0.70 g, respectively, for C + Oil and ATD + Oil).

A significant decrease in available cytoplasmic estrogen receptors was found in the epididymal fat pads of the C + 1 mg TP treatment group compared to each of the other groups, $F$ (4,39) = 8.1, $p<.00001$ ($p<.01$ all post hoc tests; Figure 2). The other four groups did

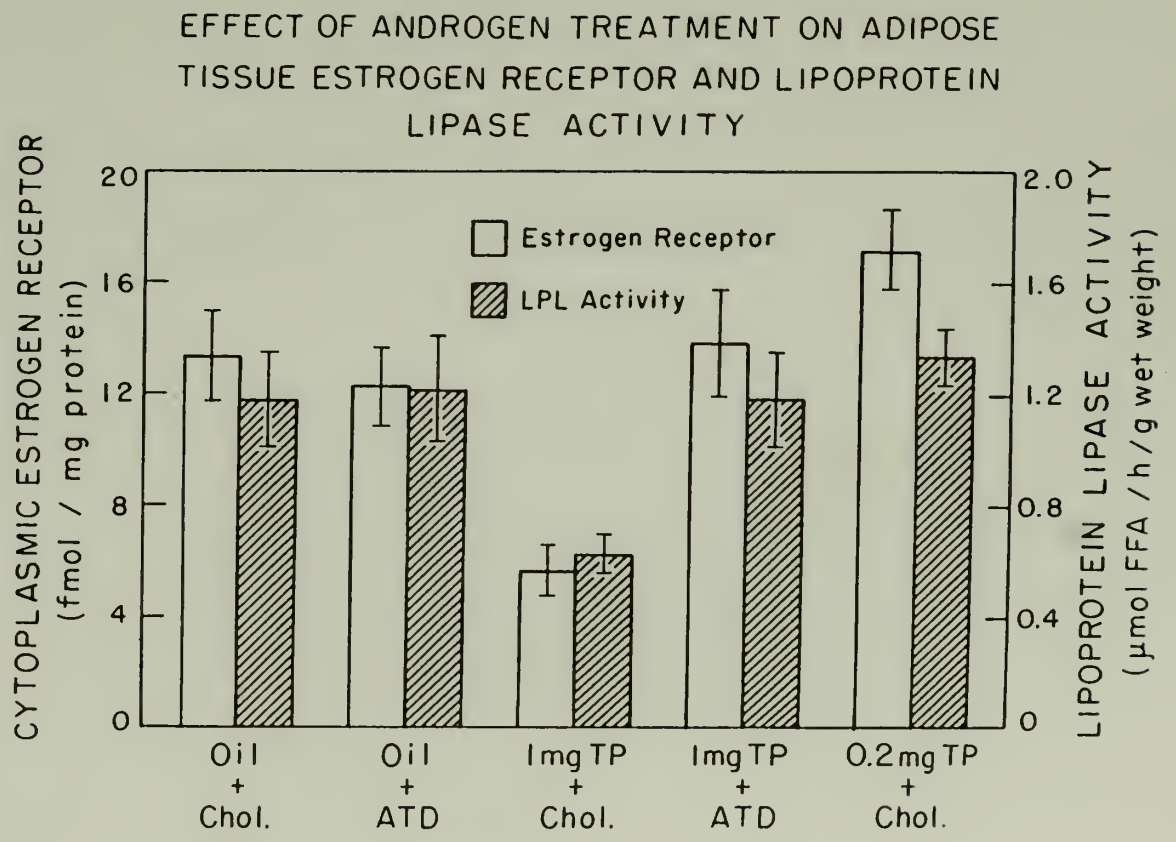



Fig. 2. Effect of TP treatment on epididymal adipose tissue estrogen receptor and lipoprotein lipase activity in GDX rats bearing Silastic implants containing cholesterol or ATD.

not differ significantly from one another on this measure. Similarly, LPL activity was significantly decreased in the C + 1 mg TP group compared to all other groups, $F$ (4,36) = 5.57, $p<.001$ ($p<.01$ all comparisons), whereas no other significant changes in enzyme activity were found between groups (Figure 2).

## Discussion

The results of Experiment I are in agreement with earlier observations of a dose-dependent effect of TP on weight gain in male rats (Gentry and Wade, 1976a; Kochakian and Endahl, 1959; Kochakian and Webster, 1958). During the first two weeks of hormone treatment all groups receiving TP gained more weight than the oil control groups. After this period weight gain slowed in the group receiving the high dose of TP + C such that by the end of the experimental period the low dose TP + C group and the 1 mg TP + ATD group had gained significantly more weight than the high TP group or oil controls. Treatment with ATD alone had no significant effect on body weight gain. Food intake was also increased by testosterone treatment. At the end of treatment all groups receiving TP were eating significantly more than were the oil control groups. Food intake of the high dose TP group was lower than that of the low TP group and the high TP + ATD group, but this

difference was not statistically significant (Table 1). These observations support the hypothesis that the dose-dependent effects of TP result from the aromatization of testosterone to estrogens (particularly estradiol) in rats treated with high doses of TP. Indeed, during the last 15 days of hormone treatment, food intake and body weight gain of rats receiving the higher doses of TP concurrently with ATD were quite similar to those of animals treated with low amounts of TP.

In the earlier phase of the experiment, food intake and body weight did not always change in parallel as one would expect if body weight gain directly reflected food intake. Whereas by day 15 all TP treatment groups had gained significantly more weight than the oil control groups, food intake during the first 15 experimental days was only elevated in the low dose TP group. As will be discussed more fully in Chapter IV, androgens have anabolic effects (Bardin, Bullock, Mills, Lin and Jacob, 1978; Kochakian, 1975); the increased weights of the TP groups might be due to the anabolic actions of the hormone. In addition, at the end of the experiment, the high dose TP group weighed significantly less than the other two TP groups, but the food intake of the high TP group did not differ significantly from that of the 1 mg TP + ATD group or the .2 mg TP + C

group. In light of the fact that androgens do have anabolic effects it is reasonable to suggest that rats might be eating for nutrients other than calories (e.g., protein). However, since no diet selection was permitted, the animals had to balance multiple metabolic needs through ingestion of a single diet. Other experiments have demonstrated that for female rats given certain gonadal hormones, food intakes and body weights need not change in parallel (Hervey and Hervey, 1968; Roy and Wade, 1977).

In Experiment II, cytoplasmic estrogen receptors and LPL activity were measured in the epididymal fat pad, an adipose tissue that has been shown to contain estrogen-specific, high affinity receptors for estradiol in male rats (Gray and Wade, 1980). Animals given high doses of TP had fewer available cytoplasmic receptors in this depot than did oil-treated control animals or than rats treated with low doses of TP. Human adipose tissues contain enzymes necessary for aromatization of androgens to estrogens (Kley et al., 1980; Nimrod and Ryan, 1975; Perel and Killinger, 1979). Thus, it is likely that in rats receiving C + 1 mg TP, some testosterone is converted to estradiol which then depletes available cytoplasmic estrogen receptors, possibly by causing translocation to cell nuclei. The estrogen

specificity of the receptor depletion is demonstrated by its absence in animals treated with 1 mg TP but given the aromatase inhibitor, ATD.

LPL activity closely paralleled the cytoplasmic estrogen receptor data (Figure 2). These results confirm and extend a previous report by Wilson and colleagues (1976) which showed that chronic treatment of male rats with low doses of testosterone (500 µg/week) had no effect on adipose tissue LPL activity, whereas estradiol administration significantly lowered activity.

Although estrogen receptors were depleted and LPL activity was decreased in rats treated with high doses of TP, mean epididymal fat pad weight for this group was not significantly lower than in rats receiving lower doses of TP. The failure to find a decrease in epididymal fat pad weight is in agreement with an earlier report by Krotkiewski (1976) that found the epididymal pad (but not other fat pads) to be spared from the anorexic effects of synthetic steroid hormone (dexamethasone) treatment. In contrast, retroperitoneal fat pad weights were lower in the high dose TP group than in all other groups.

A decrease in body weight, a depletion of adipose tissue cytoplasmic estrogen receptors and a reduction in adipose tissue LPL activity are changes seen after

treatment with estradiol. All these effects were observed in male rats given long-term treatment with high doses of TP. None of these changes were observed with low doses of TP, or when high doses of TP were administered in conjunction with the aromatase inhibitor, ATD. Taken together, these data lend strong support to the hypotheses (Gentry and Wade, 1976a; Wade and Gray, 1979) that the weight-reducing effects of high doses of TP are mediated via aromatization to estrogens, and that estradiol has a direct effect on adipose tissue metabolism, including LPL activity.

# CHAPTER III

# ANDROGEN EFFECTS ON FOOD INTAKE: CENTRAL SITES OF ACTION

## Experiment III: Effects of Diencephalic Androgen Implants on Food Intake in GDX Male Rats

Evidence supporting a direct effect of estrogens on male rat adipose tissues (Experiments I and III) does not preclude the possible involvement of other peripheral and central mechanisms in the androgenic regulation of food intake and body weight. In 1970, Wade and Zucker showed that implants of EB into the VMH in OVX rats resulted in a reduction in food intake that was similar to that seen after systemic injections of EB. Implants of this hormone in other diencephalic sites produced no reliable changes in food intake. These results have subsequently been replicated in several laboratories (Beatty, O'Briant and Vilberg, 1974; Jankowiak and Stern, 1974; Roy, Maass and Wade, 1977). Thus, estrogens may act on central as well as peripheral tissues (Wade and Gray, 1978; 1979) to affect food intake and body weight regulation.

In the present study, the possible involvement of central mechanisms in androgen-induced changes in food intake in GDX male rats were examined. Two anatomical

sites, the VMH and the preoptic-anterior hypothalamic area (POA) were directly stimulated with TP or DHTP. The VMH and POA have been shown to contain both androgen and estrogen receptors (Barley, Ginsburg, Greenstein, Maclusky and Thomas, 1975; Eisenfeld, 1970; McEwen, 1976) as well as aromatizing enzymes (Naftolin, Ryan, Davies, Reddy, Flores, Petro, White, Takoaka and Wolin, 1975). These regions have been implicated in the mediation of several of the behavioral effects of gonadal hormones (Davis and Barfield, 1979a; 1979b; Smith, Damassa and Davidson, 1977; Wade and Zucker, 1970) including eating behavior (Beatty, O'Briant and Vilberg, 1974; Jankowiak and Stern, 1974; Roy et al., 1977; Wade and Zucker, 1970).

Method.

Animals and housing. Adult male CD-strain rats (body weight 300-330 grams at the time of surgery) obtained from Charles River Breeding Laboratories were used. Rats were individually housed in hanging cages with free access to food pellets (Purina rodent chow) and tap water. A 12:12 light-dark cycle (lights on at 0800 hr) was maintained throughout the experiment.

Procedure. All animals (n = 14) were castrated via a single incision under methoxyflurane anesthesia. After a 2-week recovery period the animals were

anesthetized with sodium pentobarbital (Nembutal; 40 mg/kg) and stereotaxically implanted with bilateral, concentric double-walled stainless steel cannulae (inner 27 ga; outer 22 ga). The cannulae were aimed at the VMH (n = 7) or at the POA (n = 7). The coordinates for each placement were: VMH: anterior 5.6, lateral 1.0, vertical -3.2; POA: anterior, 7.6, lateral 1.0, vertical -2.0 (Pellegrino and Cushman, 1967).

Beginning 2 weeks after stereotaxic surgery, food intake was measured daily between 1030 and 1100 hr for the duration of the experiment. Food spillage was collected, and 24-hour food intakes were recorded to the nearest .1 g. After obtaining stable daily intakes (less than 10% variation in food intake over at least 3 days), 72-hour periods of hormone treatment were initiated. To deliver the hormone, the inner cannulae were removed and the tips (190 μm inner diameter) were filled with undiluted crystalline TP (Sigma Chemical Co.) or DHTP (Steraloids Inc.) following the procedure described by Wade and Zucker (1970). The hormone-filled cannulae were then reinserted into each animal's implanted outer cannulae where they remained for 72 hours. Inner cannulae were removed between 1100 and 1130 hr and were replaced by empty inner cannulae of identical

construction. Rats were tested with both hormones in random order. Each test was separated by at least 7 days of stable intakes. The average daily intake for the three days preceding each test was used as a baseline to assess the effects of the hormones.

Histology. At the completion of testing, all animals were given an overdose of sodium pentobarbital and perfused intracardially with 0.9% NaCl followed by 10% Formalin. Brains were removed, and frozen coronal sections were cut at 40 μm, stained with cresyl violet and examined microscopically to determine cannulae placements.

Results. An animal showing a change in mean food intake of 15% or more during the three days of hormone treatment from its prehormone baseline value (mean intake during the three days prior to hormone implantation) was considered to have a positive response to the hormone. When tested with TP, 5 of the 7 animals with implants in the VMH met this criterion (Figure 3A). Food intake of these animals during TP treatment decreased an average of 28.6% (mean intake $\pm$ S.E.M. = 71.4 $\pm$ 3.4% of baseline). Implants of TP in the POA failed to change food intake by more than 5% in any individual animal (mean = 103.4 $\pm$ 2.4%). The two groups differed significantly in responses to TP stimulation (Fisher test,

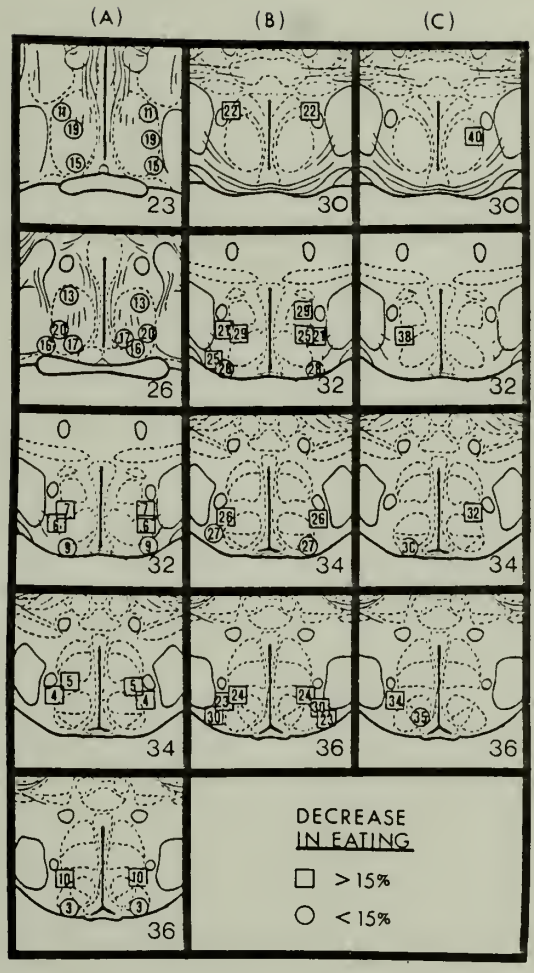
$p<0.05$, two-tailed). When DHTP was placed in the VMH or POA, no positive responses were obtained from any of the animals tested (mean for VMH implants = 101.0% $\pm$ 2.7%; mean for POA implants = 99.4 $\pm$ 1.6%). For the group with VMH implants, the effect of TP treatment was significantly different from that of DHTP (Wilcoxon matched-pairs, signed ranks test, $p<0.05$).

Implants in the VMH that resulted in positive responses to hormonal stimulation were located dorsolateral to the ventromedial nuclei. The implants for the POA group were located in the preoptic-anterior hypothalamic area lateral to the suprachiasmatic nuclei (Figure 3A).

## Experiment IV: Effects of VMH Implants of EB, TP, or DHTP on Food Intake in GDX Male Rats

In Experiment III, it was shown that bilateral implants of TP in the VMH reduced food intake in GDX male rats, whereas similar implants in the POA or VMH implants of DHTP had no effect on food intake. If TP implanted in the VMH decreases food intake via estrogenic metabolites, then EB should produce a similar decrement in food intake. In the present experiment rats were given bilateral cannulae implants in the VMH and were tested for the effects of TP, DHTP and EB on food intake. In addition, a second group of rats was

Fig. 3. Location of diencephalic hormone implants. Positive (squares) and negative (circles) sites are indicated on coronal sections adapted from König and Klippel (1963); numbers in the lower right refer to plates in the atlas. Numbers inside symbols identify individual rats. In Figure 3A (left panel) positive sites indicate responses to TP stimulation (Experiment III). In Figures 3B (middle panel) and 3C (right panel) positive sites refer to EB or TP stimulation (Experiment IV). Animals Number 26 and 32 responded to TP only; Numbers 21, 22, 25, 34, 38 and 40 responded to EB only; Numbers 24, 29 and 30 responded to both EB and TP. Numbers 23 and 36 were not tested with EB.


(A)
(B)
(C)
23
30
30
26
32
32
32
34
34
34
36
36
36
DECREASE
IN EATING
>15%
<15%

given unilateral implants in the VMH, and the effects of the above hormones on food consumption were examined.

Method.

Animals and housing. Twenty adult male CD-strain rats (weighing 300-330 grams at the time of surgery) were used. Rats were housed and maintained as were the animals in Experiment III.

Procedure. Rats were castrated as previously described. Following the procedures described in Experiment III, rats were given unilateral (n = 10) or bilateral (n = 10) implants of concentric cannulae aimed at the VMH. Rats were tested with TP, DHTP and EB as described in Experiment III. The histological procedures used were those described in Experiment III.

Results. Bilateral implants of TP in the VMH reduced food intake by more than 15% from baseline in 5 of 10 animals (mean = 75.8 $\pm$ 2.2%). None of the rats in this group changed its mean intake by more than 7% when tested with DHTP (mean = 100.5 $\pm$ 1.2%). Nine of these 10 animals were also tested with EB and 6 responded with reductions in food intake that were greater than 15% (mean = 76.3 $\pm$ 3.3%). Three of the 6 EB-responders had shown similar decreases in food intake in response

to TP, the other 3 animals responded to EB but not to TP (Figure 3B). The effects of TP and EB were significantly different from the effect of DHTP on food intake (Chi square test, $p<0.01$ in both cases).

Of the 9 animals with unilateral VMH implants, 3 lost their implants shortly after the first hormonal test and were excluded from the experiment. Of the remaining 6 rats, only 1 responded to TP (20.7% reduction), and 3 responded to EB (mean = 76.3 $\pm$ 1.8%). Treatment with DHTP did not affect food intake in 5 animals (mean 96.5% $\pm$ 2.8%). However, 1 animal showed a 16.2% reduction during DHTP treatment, and this animal (Number 35, Figure 3C) failed to respond to TP and EB. Differences between treatments were not statistically significant (Chi square test, $p>0.05$).

As in Experiment III, most of the effective implants were located dorsolateral or lateral to the ventromedial nuclei (Figure 3B and 3C).

For all animals in the study (as well as in Experiment III), effective implants had their most pronounced effect during the first 24 hours of hormonal stimulation. In almost all cases, the animals returned to pretreatment baselines within three days after the removal of the hormone. For 3 animals, food intake stabilized at a lower baseline (10-12% reduction) after

a positive response to the treatment.

## Discussion

Castrated male rats given peripheral injections of TP in high doses show an initial increase followed by a permanent reduction in food intake and body-weight gain compared to rats given lower doses of the hormone (Experiment I; Gentry and Wade, 1976a). It has been proposed that hormone-induced changes in LPL activity may be partly responsible for some of the effects of high doses of TP on food intake, body weight and adiposity. The present results suggest that in addition to its peripheral effects, TP may act directly in the hypothalamus to influence food intake in male rats. These observations may reflect a direct effect on feeding behavior or a change in metabolism that indirectly affects food intake. In this as well as other studies (Beatty et al., 1974; Jankowiak and Stern, 1974; Roy et al., 1977; Wade and Zucker, 1970), the implants that were effective in changing food intake were located in the VMH. This neural area has long been implicated in the control of feeding behavior (Grossman, 1975; Panksepp, 1974) as well as in the modulation of endocrine and autonomic-nervous system functions that may alter metabolism (Bray, 1974; Bray and Nishizawa,

1978; Gisel and Innes, 1979) and thereby indirectly affect feeding behavior (Friedman and Stricker, 1976).

As shown in Chapter I, some of the effects of TP on adipose tissues seem to be mediated by estrogenic metabolites of the hormone. A similar mechanism may also be responsible for the central effects of TP on food intake. That hypothalamic implants of EB reduce food intake in male rats (Beatty et al., 1974) lends credence to this view. In addition, in the present experiments, EB treatment tended to be more effective than TP in reducing food intake, whereas treatment with DHTP, a non-aromatizable androgen, failed to have any effect on this measure. It is interesting to note that the positive anatomical sites in the present study were very similar in location to those reported for female rats (Beatty et al., 1974; Jankowiak and Stern, 1974; Roy et al., 1977; Wade and Zucker, 1970). Here again, as in the case of feminine sexual behavior (Davis and Barfield, 1979), estrogenic stimulation of VMH produces quite similar responses in male and female rats (Beatty et al., 1974).

It cannot be assessed from the present data whether any of the hormones administered migrated to sites other than the VMH. However, implants in the anterior hypothalamus and preoptic area were ineffective

in reducing food intake, suggesting some degree of anatomical specificity of the hormone effect. Furthermore, the effects were evident within the first 24 hours of hormone administration. Thus, the hormone is probably acting on neural tissue near the site of the implant. It is also unlikely that any of the reported effects are the result of leakage of the hormone to the periphery, since systemic administration of TP, even in high doses, produces an initial increase in food intake (Experiment I; Gentry and Wade, 1976a). The rapid reduction in food intake seen after effective TP implants argues against a peripheral effect. Finally, unilateral implants of EB in the VMH reduce food intake in female rats, but have no effect on cytoplasmic estrogen receptor concentration or LPL activity in adipose tissues in these animals (Nunez, Gray and Wade, 1980). These data suggest that estrogenic stimulation of the VMH (independent of any peripheral effects of the hormone) may be sufficient to produce hypophagia, and support the notion that the effects observed in Experiments III and IV are not the result of leakage of the hormones to non-neural sites.

# CHAPTER IV

# EFFECTS OF ANDROGENS ON DIETARY SELF-SELECTION, BODY WEIGHT AND CARCASS COMPOSITION IN CASTRATED MALE RATS

While it is known that ovarian hormones alter selection of dietary components by rats (Leshner and Collier, 1973; Leshner, Siegel and Collier, 1972; Richter and Barelare, 1938; Wurtman and Baum, 1980), little is known about the role of androgens in determining choice of dietary constituents. In the following studies, the dietary self-selection patterns of GDX males receiving one of two doses of either TP or DHTP were examined. The effects of these hormones on carcass composition were also investigated. The analysis of carcass composition was of interest because 1) it was not known which carcass components were affected during weight gain that is induced by chronic administration of low doses of TP, and 2) the effects of a non-aromatizable androgen (DHTP) on carcass composition had not been studied.

## Experiment V: Testosterone and Protein Intake

As discussed in preceding chapters, several lines of research suggest that the lowered body weights of males given high doses of TP are due, at least in part, to

metabolic effects of estrogenic metabolites of the hormone (Gentry and Wade, 1976a; Hervey and Hutchinson, 1972; Kochakian, 1950). The mechanism by which testosterone increases body weight is less clear.

It is known that testosterone, primarily via its 5α-reduced metabolite, DHT, stimulates growth in genital tissues containing specific cytoplasmic androgen receptors (and the enzyme 5α-reductase), such as prostate and epididymis (Liang et al., 1977; Mainwaring, 1977). Yet, the testosterone-induced gain in body weight is larger than that which can be accounted for by growth of reproductive tissues (Kochakian, 1946). In addition to its androgenic action in accessory sex organs, testosterone increases nitrogen retention and deposition of protein in other peripheral tissues (Kochakian, 1975; Rogozkin, 1979). These anabolic actions have been demonstrated in tissues containing cytoplasmic androgen receptors, but which have little or no 5α-reductase activity (i.e., skeletal muscle and kidney; Bardin et al., 1978; Krieg and Voigt, 1977; Mainwaring, 1977); suggesting that unmetabolized testosterone (or some testosterone metabolite other than DHT) may itself have anabolic effects.

That testosterone has marked protein anabolic effects is of interest in light of the finding that

intact male rats consume a larger percentage of their diets as protein when allowed to self-select dietary components than do self-selecting female rats (Leshner and Collier, 1973). As suggested earlier (Chapter II), the increase in food intake in testosterone-treated, GDX male rats might reflect an increase in consumption of a particular nutrient such as protein. However, because rats in that experiment were fed standard laboratory chow, and thus could only increase protein intake by increasing ingestion of the entire diet, this hypothesis could not be tested. An extensive body of literature has established that rats do have the capacity to regulate protein consumption independently from total energy intake under a wide range of varying physiological and environmental conditions (Anderson, 1979; Booth, 1974; Collier, Leshner and Squibb, 1969a; 1969b; Leshner, Collier and Squibb, 1971; Wurtman and Baum, 1980).

Experiment V was designed to test the influence of TP on intake of dietary components. Castrated adult male rats, each receiving a daily injection of either a high or low dose of testosterone, or the injection vehicle alone, were given access to a pair of isocaloric diets that were equal in fat content, but that contained differing amounts of protein and

carbohydrate.

## Method

Animals and housing. Sixty adult male CD-strain rats were purchased from Charles River Breeding Laboratories. They were housed singly in a room providing 12 hours of illumination (lights on at 2400 hr). Rats were gonadectomized under methoxyflurane anesthesia via a single scrotal incision. Animals were given unlimited access to tap water throughout the experiment, and food pellets (Purina laboratory chow) were provided ad libitum until the introduction of the experimental diets.

Four weeks after orchiectomy, rats were divided into two groups that were matched in terms of body weight (n = 30) each. One group (mean body weight $\pm$ S.E.M. = 313.9 $\pm$ 3.9 g) was assigned to the diet conditions given below, and the other was assigned to the conditions of Experiment VI.

Diets. Both diets contained 4% (by weight) Hegsted salt mixture (ICN Pharmaceuticals), 1.5% Vitamin Diet Fortification Mix (ICN) and 20% vegetable shortening. The low protein diet (Diet A) contained 5% of the high quality protein, casein (purified high nitrogen; ICN),

and 69.5% carbohydrate as cornstarch. The high protein diet (Diet B) included 40% casein and 34.5% cornstarch. The diets were isocaloric (4.8 kcal/g).

Procedure.

Adaptation to diets. Four weeks after castration, food pellets were removed from all cages, and two 8.5 cm high X 7.0 cm diameter glass jars, each containing one diet of the diet pair, were attached with springs to the inside of each cage. Animals were allowed 2 weeks to adapt to the new diets. If an animal showed a strong preference for one of the diets during this time, the diet was removed temporarily so that the rat could gain experience with the other diet of the pair. Prior to the collection of baseline data, all animals were eating from both food jars. The positions of the food jars were varied throughout the adaptation and experimental periods.

Measurement of food intake and body weight. After adaptation to the diets, all rats received daily subcutaneous injections of .1 ml sesame oil. Beginning 1 day after the start of oil injections, food intake (to the nearest .1 g) and body weight (to the nearest g) were measured every third day approximately 2 hours before lights out. After one week of oil

injections, animals were matched for body weight, total food intake and protein intake, and divided into three hormone treatment groups (n = 10 each). Rats subsequently received injections of either 2.0 mg TP or .2 mg TP in .1 ml sesame oil, or .1 ml of the vehicle alone.

Throughout the experiment food spillage was minimal for most rats. However, after several weeks of data collection, one subject in the oil group and one rat in the .2 mg TP group consistently spilled portions of both diets. All data obtained from these animals were discarded. Even without these animals the three groups were closely matched for body weight and consumption of the two diets (Table 2). Analysis of variance indicated no significant differences on any of the dependent measures.

Carcass analysis. At the end of the experiment, all animals were given an overdose of sodium pentobarbital and promptly shaved and eviscerated in preparation for carcass analysis. Carcass composition was determined by a modification of the method of Leshner, Litwin, and Squibb (1972). The eviscerated carcasses were baked at 70° - 90°C until no further weight losses were noted (2 - 3 weeks). Each dried carcass was homogenized in

TABLE 2

BASELINE BODY WEIGHT AND INTAKE OF DIETARY COMPONENTS IN RATS FED DIETS A AND B (Mean ± S.E.M.)

| GROUP | BODY WEIGHT (grams) | TOTAL FOOD (kcal/day) | PROTEIN (kcal/day) | CARBOHY-DRATE (kcal/day) |
|---|---|---|---|---|
| Oil | 370.4 ± 9.7 | 90.8 ± 3.9 | 17.0 ± 2.7 | 39.4 ± 2.7 |
| 0.2 mg TP | 369.3 ± 7.9 | 91.8 ± 2.8 | 17.3 ± 1.8 | 39.7 ± 2.0 |
| 2.0 mg TP | 371.3 ± 7.6 | 90.2 ± 2.6 | 17.6 ± 2.0 | 38.4 ± 2.0 |

a Waring blender and mixed well to insure homogeneity. Approximately 0.5 g of the homogenate was used for determination of lipid content. Lipid was extracted with 2 x 10 ml petroleum ether. Dried-delipidated samples were dissolved in .3 N KOH, and protein content was determined by the method of Lowry, Rosebrough, Farr and Randall (1951).

Data analysis. All food intake data were converted to calories (based on protein = 4 kcal/g; carbohydrate = 4 kcal/g; fat = 9.1 kcal/g). Separate analyses of variance were performed on total food, protein, carbohydrate and fat intake over the entire 27-day period. Since earlier experiments have shown that body-weight gain and food intake of rats receiving a high dose of TP increase before the attenuating effects of the TP are observed, post-baseline data were divided into three

blocks of 9 days each for further analysis. Separate analyses of variance were then performed on each of the (above) dependent measures at each 9-day block. Analyses of variance were followed by individual comparisons of means using Newman-Keuls tests (Winer, 1962). Body weight data were expressed as percentage change from baseline and were subjected to analysis of variance followed by Newman-Keuls tests.

## Results

Total caloric intake. Testosterone propionate increased total caloric intake throughout the 27 days of treatment, $F$ (2,25) = 15.05, $p<.0001$ (Figure 4). Over the entire injection period, the mean caloric intake of both the high TP (mean $\pm$ S.E.M. = 89.7 $\pm$ 1.9 kcal/day) and low TP (97.2 $\pm$ 1.0 kcal/day) treatment groups differed significantly from that of the oil-treated group (81.4 $\pm$ 2.7 kcal/day; $p<.01$, both tests). In addition, the group receiving .2 mg TP consumed more calories than did the group receiving 2.0 mg TP ($p<.05$).

During the first 9 days, total caloric intake rose in both groups receiving TP, $F$ (2,25) = 3.41, $p<.05$ (Figure 4). Total food intake during this time was significantly higher in the low TP group than in the

oil-treated controls ($p<.05$). The group receiving 2.0 mg TP did not differ from either the low TP or oil-treated groups. Food intake was also significantly increased by TP administration during days 10-18, $F$ (2,25) = 10.94, $p<.0004$, and days 19-27, $F$ (2,25) = 17.30, $p<.00002$ (Figure 4). Individual comparisons indicated that during days 10-18, the high and low TP groups ate significantly more than the control group ($p<.05$, $p<.01$, respectively); and the .2 mg TP-treated animals consumed significantly more than did the 2.0 mg TP group ($p<.05$). These differences were also present during days 19-27 ($p<.01$, all tests).

Protein intake. Protein intake was increased in both TP-treated groups throughout the 27-day experimental period, $F$ (2,25) = 11.94, $p<.0001$ (Figure 4). The mean protein intake of both the .2 mg TP group (mean $\pm$ S.E.M. = 18.2 $\pm$ 1.0 kcal/day) and the 2.0 mg TP group (19.2 $\pm$ .9 kcal/day) differed significantly from that of the controls (13.0 $\pm$ .9 kcal/day); while the two groups receiving TP did not differ significantly from one another.

That hormone treatment affected protein intake at all three periods was confirmed by separate analyses on data from days 1-9, $F$ (2,25) = 5.75, $p<.01$; days 10-18,

$F$ (2,25) = 7.7, $p<.003$; and days 19-27, $F$ (2,25) = 12.32, $p<.0002$). Post hoc tests indicated that protein consumption was significantly higher for both groups receiving TP compared to the oil control group ($p<.05$ for low TP group, days 1-9; $p<.01$ for all other comparisons). The .2 mg TP and 2.0 mg TP groups did not differ significantly during any of the 9-day blocks.

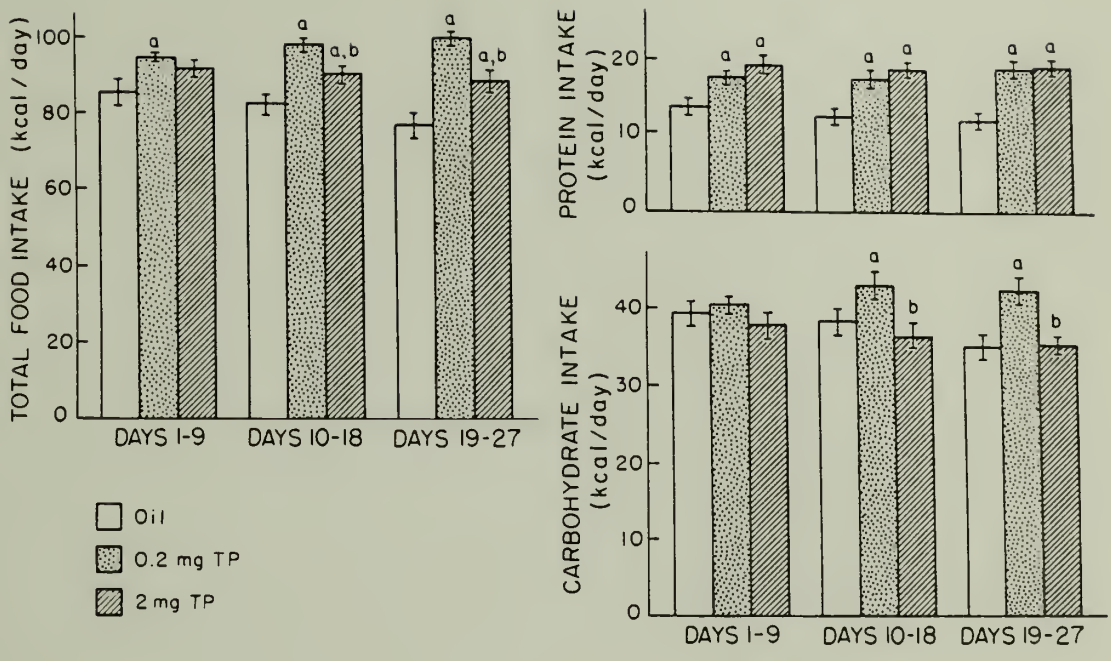
Carbohydrate intake. Carbohydrate intake was increased by the low dose of TP and was relatively unaffected by the high TP dose, $F$ (2,25) = 5.86, $p<.008$ (Figure 4). The mean number of calories consumed as carbohydrate during the 27 treatment days was significantly higher in the .2 mg TP group (mean $\pm$ S.E.M. = 42.1 $\pm$ 1.2 kcal/day) compared to either the 2.0 mg TP group (36.5 $\pm$ 1.1 kcal/day) or the oil-treated control group (37.5 $\pm$ 1.4 kcal/day; $p<.05$, both tests). Carbohydrate intake did not differ significantly between the high TP dose group and the oil-injected group.

Carbohydrate intake was not affected by hormone treatment during the first 9 days, $F$ (2,25) = .88, $p = .43$. However, the effect of TP was evident by days 10-18, $F$ (2,25) = 4.55, $p<.02$, and was still present during the last third of the experiment, $F$ (2,25) = 8.35, $p<.002$. The low TP group consumed significantly more

Fig. 4. Effects of high or low doses of TP or injection vehicle alone on total food (left panel), protein (upper right) and carbohydrate (lower right) intake in GDX rats fed Diets A and B. Rats were allowed to self-select from two isocaloric diets that varied in amounts of protein and carbohydrate. Data are presented as means ± S.E.M.

[a]Significantly different from oil-injected group.

[b]Significantly different from group receiving .2 mg TP.


TOTAL FOOD INTAKE (kcal / day)
0
20
40
60
80
100
a
a
a,b
a
a,b
DAYS 1-9
DAYS 10-18
DAYS 19-27
Oil
0.2 mg TP
2 mg TP
PROTEIN INTAKE (kcal/day)
0
10
20
a
a
a
a
a
a
CARBOHYDRATE INTAKE (kcal/day)
0
10
20
30
40
a
b
a
b
DAYS 1-9
DAYS 10-18
DAYS 19-27

calories as carbohydrate than did the high TP group or the oil controls during days 10-18 ($p<.05$, both tests) and during days 19-37 ($p<.01$, both tests).

Fat intake. Since both the diets in this experiment were isocaloric and each contained 20% fat, fat intake varied as a function of total caloric intake and accounted for 37.9% of the total calories consumed.

Body weight. Testosterone propionate increased body weight gain during the first 9 days of treatment, $F$ (2,25) = 17.59, $p<.00002$ (Figure 5). Mean weight gain of the two TP-treated groups was nearly identical at day 9 of treatment, and thus did not differ significantly from one another. However, both groups gained significantly more weight than did the controls ($p<.01$, both TP groups).

After the ninth day of treatment, body weight continued to rise in the group receiving .2 mg TP, while weight gain began to slow in the 2.0 mg TP group. An analysis of variance at day 18 indicated a significant treatment effect, $F$ (2,25) = 17.10, $p<.00002$. Post hoc tests revealed that body weights were significantly higher in the two TP treatment groups compared with the oil-treated group ($p<.01$, both TP groups), but the .2 mg TP group weighed significantly

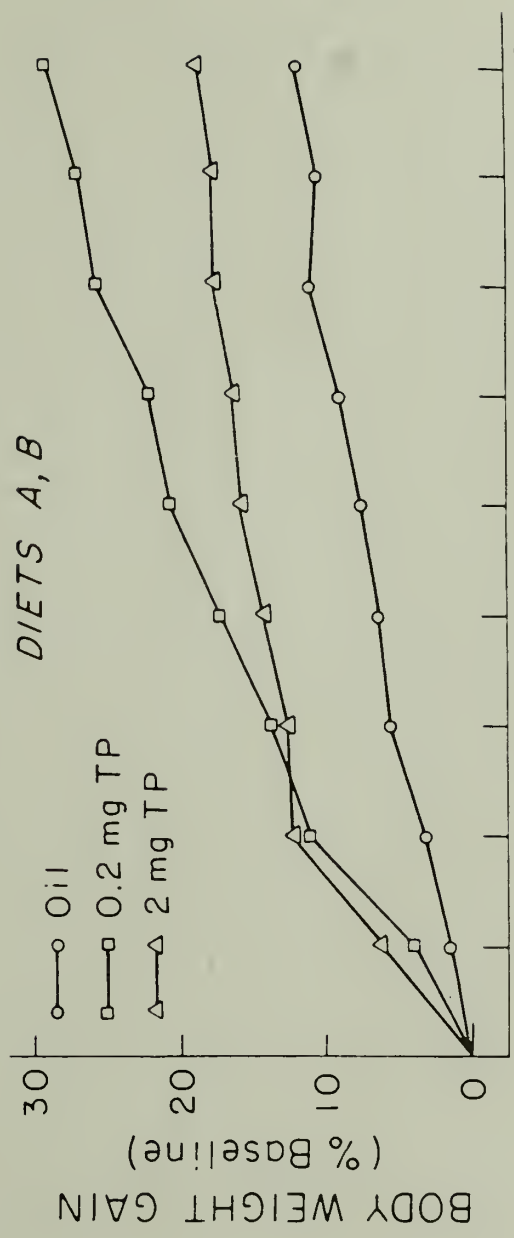



Fig. 5. Effects of long-term TP treatment or oil injection vehicle on body weight gain in GDX rats self-selecting from Diets A and B.

more than the 2.0 mg TP group ($p<.05$).

At the end of the 27-day treatment period, body weight gain had slowed greatly in the high TP group, while the low TP group continued to gain rapidly, $F$ (2,25) = 15.07, $p<.00005$. By day 27, the group receiving .2 mg TP weighed significantly more than the oil ($p<.01$) and 2.0 mg TP group ($p<.01$). The high TP group was not significantly different from controls. The body weights (mean $\pm$ S.E.M.) of the oil, low TP and high TP groups at day 27 were, respectively, 415.7 $\pm$ 9.8 g, 474.3 $\pm$ 7.1 g and 438.8 $\pm$ 11.2 g.

Carcass composition. Carcass analyses are reported in Experiment VI.

## Discussion

The results of Experiment V extend those of earlier studies which reported a dose-dependent effect of TP on body weight gain and food intake in castrated male rats (Experiment I; Gentry and Wade, 1976a; Kochakian and Endahl, 1959; Kochakian and Webster, 1958). Low doses of TP increased body weight gain and total food intake more than did higher doses of TP.

It was proposed that TP-treated rats might increase food intake to acquire a greater quantity of some specific macronutrient (e.g., protein). The

present data suggest that this is not the case for self-selecting GDX rats given long-term treatment with .2 mg TP. The increased caloric intake of these animals was due to enhanced consumption of both carbohydrate and protein. However, it should be noted that during the first 9 treatment days, protein intake was significantly elevated in the low TP group, whereas calories consumed as carbohydrate did not differ from the vehicle-treated control animals.

In contrast, injections of 2.0 mg TP had a striking effect on intake of a single nutrient, protein. Beginning after the ninth day of treatment, total caloric intake in the high TP group decreased significantly compared with the low TP group, yet consumption was still significantly higher than that of the controls. At the same time, the number of calories ingested as carbohydrate were nearly identical to that of controls. However, protein intake was significantly elevated in the high-dose TP group compared to the controls throughout the experiment. Thus, the increased food intake in the 2.0 mg TP group observed during the last 18 days of treatment was due largely to enhanced ingestion of protein. That calories ingested as carbohydrate were similar in the high TP and oil groups suggests that the increased protein intake of

the high TP group was not due to an attempt to avoid carbohydrate. Thus, high doses of TP increase protein intake even when given for a sufficient length of time to cause a decline in body weight gain.

## Experiment VI: Testosterone and Carbohydrate Intake

In Experiment V, the low (5%) protein diet contained 69.5% carbohydrate, and the high (40%) protein diet contained 34.5% carbohydrate. Thus, enhanced consumption of protein and decreased ingestion of carbohydrate might both be manifest as an increase in calories eaten as protein. To rule out the possibility that TP-treated animals were eating protein to avoid carbohydrate, a second group of rats was given access to a pair of diets that had equal percentages of protein and of fat, but that contained either a high or low percentage of carbohydrate. As in Experiment V, rats each received a daily injection of either .2 mg TP, 2.0 mg TP or the injection vehicle alone.

### Method

Subjects. Subjects were thirty CD-strain rats. Animals were housed and fed as in Experiment V.

Diets. Each diet contained 4% (by weight) Hegsted Salt

Mixture, 1.5% Vitamin Diet Fortification Mix, 20% vegetable shortening, and 25% casein. Diet C contained 49.5% cornstarch as carbohydrate (caloric density of the diet = 4.8 kcal/g). The carbohydrate content of the second diet, Diet D, was diluted to 25% by the addition of 24.5% nonnutritive cellulose (hydrolyzed Alphacel, ICN Pharmaceuticals; total caloric content = 3.82 kcal/g).

Procedure. Experiment VI was run simultaneously with Experiment V. The procedure was identical to that of Experiment V. At the beginning of the diet adaptation period, the mean body weight ± S.E.M. of this group was 312.9 ± 3.9 g.

Prior to the completion of the experiment, food spillage was detected under the cages of two rats in the .2 mg TP group, and all data for these rats were discarded. Even without these two animals the three groups were closely matched for body weight and consumption of the two diets (Table 3).

Carcass composition and data were analyzed as in Experiment V.

## Results

Total caloric intake. Testosterone propionate increased

TABLE 3

BASELINE BODY WEIGHT AND INTAKE DATA FOR RATS FED DIETS C AND D
(Mean ± S.E.M.)

| GROUP | BODY WEIGHT (grams) | TOTAL FOOD (kcal/day) | PROTEIN (kcal/day) | CARBO-HYDRATE (kcal/day) |
|---|---|---|---|---|
| Oil | 359.8 ± 4.9 | 82.7 ± 3.8 | 17.8 ± 2.4 | 33.6 ± 5.1 |
| 0.2 mg TP | 364.0 ±12.6 | 83.4 ± 4.4 | 17.8 ± 2.6 | 33.2 ± 6.1 |
| 2.0 mg TP | 359.7 ± 9.8 | 85.8 ± 4.4 | 18.3 ± 2.9 | 33.3 ± 5.2 |

total caloric intake, $F$ (2,25) = 5.40, $p<.01$ (Table 4). Over the 27-day injection period, mean caloric intake was significantly higher in each of the groups receiving TP compared to the oil-treated control animals ($p<.05$, both groups, Table 4).

The hyperphagia-inducing effect of TP was evident in both TP groups by the ninth day of treatment (both TP groups ate approximately 8% more than controls), although the groups did not differ significantly in the first 9-day block, $F$ (2,25) = 1.83, $p$ = .18. Mean daily intakes for the .2 mg TP and 2.0 mg TP groups were 20% and 11% greater, respectively, than controls during days 10-18, $F$ (2,25) = 4.85, $p<.017$. However, during this period, only the group receiving .2 mg TP differed significantly from the oil-treated controls ($p<.05$). Mean caloric intake during days 19-27 was increased by 25%

in the low TP group and by 22% in the high TP group compared with controls, $F$ (2,25) = 7.86, $p<.002$. The caloric intake of each TP group during this last 9-day block was significantly higher than that of controls ($p<.01$, both groups).

TABLE 4

EFFECTS OF LONG-TERM TP TREATMENT ON K CALORIES OF DAILY FOOD, PROTEIN, AND CARBOHYDRATE INTAKE IN RATS FED DIETS C AND D
(Mean ± S.E.M.)

| TREATMENT | N | TOTAL FOOD | PROTEIN | CARBOHYDRATE |
|---|---|---|---|---|
| Oil Vehicle | 10 | 76.4 ± 2.2 | 16.6 ± .4 | 29.5 ± 1.3 |
| 0.2 mg TP | 8 | 89.8 ± 3.4[a] | 19.6 ± .8[a] | 34.6 ± 1.3[a] |
| 2.0 mg TP | 10 | 87.1 ± 3.4[a] | 18.9 ± .7[a] | 33.7 ± 1.4[a] |

[a]Significantly different from oil-injected group.

Protein intake. The mean number of calories consumed as protein were also increased by long-term TP treatment, $F$ (2,25) = 5.78, $p<.009$ (Table 4). Overall, mean protein intake was significantly increased by TP whether given in .2 mg ($p<.01$) or 2.0 mg ($p<.05$) doses (Table 4).

During the first 9 days of treatment, mean daily protein intakes for the low TP and high TP groups were,

respectively, 8% and 7% higher than that for controls (groups did not differ significantly during this time, $F$ (2,25) = 1.47, $p$ = .25). Again, an augmentation of the hormone effect was seen over time such that mean daily protein consumption during days 10-18 was increased about 21% in the low TP group and 12% in the high TP group, relative to controls, $F$ (2,25) = 6.05, $p<.007$. The protein intake of the low TP group was significantly different from that of controls ($p<.01$) during this period, while that of the high TP group just fell short of significance (q .95(2,25) = 18.56; difference between mean daily intake for the high TP group and oil controls = 18.27). During the last 9 days of hormone treatment, the .2 mg TP-treated rats were eating 24% more protein calories and the 2.0 mg TP group 23% more calories as protein than the oil-treated rats, $F$ (2,25) = 8.30, $p<.002$. The mean daily protein intake of each of the two TP-treated groups during the last 9 treatment days, differed significantly from that of the oil-treated controls ($p<.01$, both groups).

Carbohydrate intake. Overall, carbohydrate intake was increased by TP treatment, $F$ (2,25) = 4.36, $p<.024$ (Table 4). Intake over the 27 day experimental period was significantly elevated in both TP-treated groups

compared to the oil-treated group ($p<.05$, both groups; Table 4).

Again, TP enhanced carbohydrate consumption at all three 9-day blocks. Compared to controls, mean carbohydrate intake during days 1-9 was increased by 2% and 12%, respectively, in the low TP group and high TP group, although groups did not differ significantly in this first 9-day block, $F$ (2,25) = 2.05, $p$ = .15. During days 10-18, carbohydrate intake was 18% and 10% higher, respectively, for the low TP- and high TP-treated groups compared with controls. As in the first block, the groups did not differ significantly from one another during this time period, $F$ (2,25) = 3.16, $p$ = .06. Mean carbohydrate intake during days 19-27, was 26% and 22% higher, respectively, in the low and high TP-treatment groups compared with the vehicle-injected group, $F$ (2,25) = 6.29, $p<.006$. The carbohydrate intake of the two TP-treatment groups differed significantly from that of the control group ($p<.01$, both groups), during this last 9-day injection period.

Fat intake. Since food intake was increased by TP treatment, fat intake was also increased. The intake of fat varied as a function of calories eaten as protein. Thus, analyses are significant as for protein intake (e.g., groups differed significantly over the

27-day period, $F$ (2,25) = 5.78, $p<.009$.

Body weight. During the first nine days of treatment, testosterone increased body weight gain to levels significantly greater than controls, $F$ (2,25) = 40.29, $p<.0001$, in both the high ($p<.01$) and low ($p<.01$) dose groups (Figure 6). At the end of the injection period, mean body weight gain differed among the groups, $F$ (2,25) = 28.8, $p<.00001$. Weight gain was greatest in the .2 mg TP group, and it was significantly greater than that of the oil-treated group ($p<.01$) and the group receiving the high dose of TP ($p<.01$). Unlike the animals in Experiment V, the high TP-dose group gained significantly more than controls ($p<.01$) during 27 days of hormone treatment. At the end of the experiment, the mean body weights ($\pm$ S.E.M.) of the oil, low TP and high TP groups were, respectively, 402.0 $\pm$ 6.3 g, 457.4 $\pm$ 19.7 g, and 430.0 $\pm$ 13.0 g.

Carcass composition. Individual $t$-tests indicated that diet condition (Diets A,B versus Diets C,D) had no effect on carcass composition within each hormone treatment group. For further statistical analyses, animals receiving the same hormone treatment in Experiment V and Experiment VI were combined.

The high dose of TP significantly increased the percentage of water in the carcass, $F$ (2,52) = 5.16,

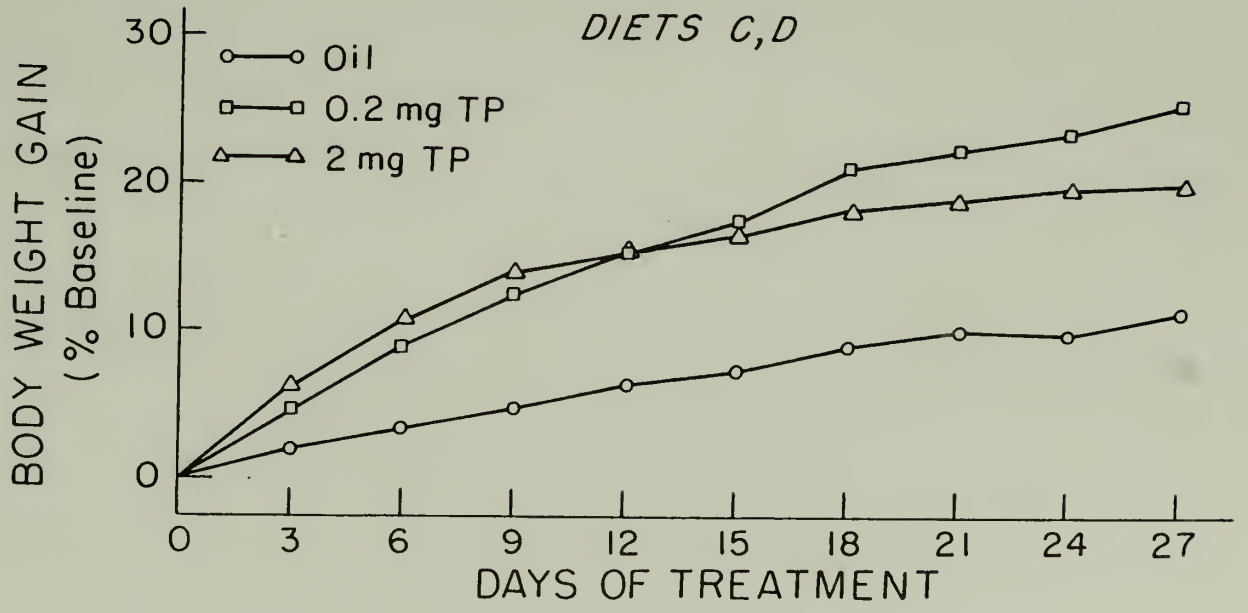



Fig. 6. Effects of long-term TP treatment or injection vehicle on body weight gain in GDX rats self-selecting from Diets C and D. Rats had access to two 25% protein diets containing differing amounts of carbohydrate.

$p<.01$), compared to both the low dose ($p<.05$) and the oil treatment ($p<.05$; Figure 7). The percentage of carcass lipid was decreased by long-term administration of 2.0 mg TP, $F$ (2,52) = 4.15, $p<.02$. Carcass lipid was significantly lower in this group compared with the .2 mg TP and oil groups ($p<.05$, both tests). The animals receiving .2 mg TP did not differ significantly from controls (Figure 7). The percentage of the carcass that was protein was not significantly affected by hormone treatment, $F$ (2,52) = 1.53, $p$ = .23.

## Discussion

When TP-treated rats were given an opportunity to select fewer calories as carbohydrate, carbohydrate intake did not decline; instead both doses of TP increased carbohydrate intake relative to controls. Thus, these data lend additional support to the contention that the increased protein intake of males given long-term treatment with high doses of TP (Experiment V, Figure 4) is not due to an attempt to avoid carbohydrate.

The increased body weight of the rats given the low dose of TP is due to increases in all carcass components. Testosterone has marked protein anabolic actions on several peripheral tissues, especially skeletal muscle. Following testosterone administration,

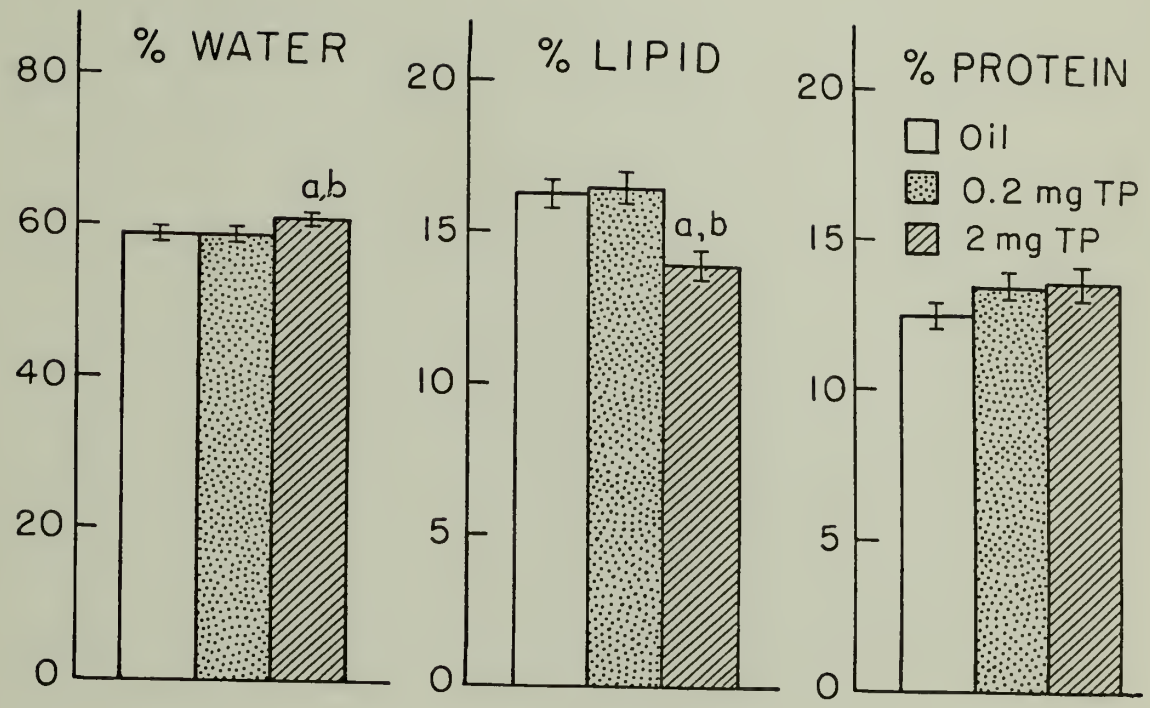



Fig. 7. Carcass composition of GDX male rats given long-term treatment with high or low doses of TP or the injection vehicle alone. Data are presented as means ± S.E.M.

[a]Significantly different from oil-injected group.

[b]Significantly different from group receiving .2 mg TP.

protein intake rises in GDX male rats; perhaps as a way of ensuring that adequate amounts of amino acids will be available for increased protein synthesis. The anabolic effects of TP can also be seen in the carcasses of TP-treated rats. The mean percentage of protein in the carcasses of these males is not significantly higher than the percentage of protein found in the carcasses of vehicle-treated males, but the absolute amount of protein in the carcasses of TP-treated rats was significantly higher than in the oil-injected animals, $F$ (2,25) = 5.43, $p$ = .007. The mean number of grams of protein in the carcasses of rats treated with either the low TP dose (59.5 $\pm$ 2.5) or the high TP dose (55.9 $\pm$ 2.8) was significantly greater ($p<.01$, $p<.05$, respectively) than that of the control rats (47.9 $\pm$ 2.0).

Finally, whether rats were allowed to self-select from diets containing 5% or 45% protein, or from 25% protein, diets had no influence on carcass composition. This finding is in agreement with data from other studies in which rats were allowed to self-select dietary constituents (Leshner, 1972; Leshner, Siegel and Collier, 1972). The protein anabolic activity of TP (as estimated by nitrogen retention) cannot be altered by increasing the protein content of the diets

(diets ranged from 18-43% protein; Kochakian and van der Mark, 1952). Apparently once adequate amounts of dietary nutrients are supplied, the proportions of the various carcass components remain unaltered.

## Experiment VII: Dihydrotestosterone and Protein Intake

Many androgenic responses in the accessory sex organs of male rats are mediated by the intracellular formation of DHT from testosterone, and its subsequent binding to specific receptors (Liang et al., 1977). Numerous studies have documented the growth-promoting effects of DHT in prostate and other genital tissues (Mainwaring, 1977). However, DHT has little anabolic action, and skeletal muscle lacks 5α-reductase, the enzyme necessary for formation of DHT from testosterone (Krieg and Voigt, 1977). Furthermore, any DHT formed in muscle is rapidly metabolized to androstanediols (Bardin and Catterall, 1981).

Like TP, DHTP also stimulates food intake and body weight gain in GDX male rats (Gentry and Wade, 1976a), but the magnitude of these DHTP effects are much smaller than those observed after TP administration. In addition, high doses of DHTP do not reduce weight gain (unlike TP), likely because DHTP cannot be aromatized to estrogens (Gentry and Wade, 1976a). Thus,

because of its growth promoting actions in some peripheral tissues and its influence on body weight, it was of interest to investigate the effects of DHTP on intake of dietary components and body composition in GDX male rats.

## Method

Animals and housing. Subjects were 30 CD male rats. Animals were housed, fed and castrated as in Experiment V, except that lights were on from 2400 hr to 1200 hr.

Diets. The experimental diets were diets A and B used in Experiment V.

Procedure.

Adaptation to diets. Four weeks after castration, rats were placed on the experimental diets (as in Experiment V) for a period of 1 week. Adaptation was as in Experiment V.

Measurement of food intake and body weight. After adaptation to the diets, all rats were given daily subcutaneous injections of .1 ml sesame oil. Food intake and body weight were measured twice weekly. After 7 days of oil injections, rats were assigned to one of three treatment groups (n = 10 each group) matched for body weight and total food, protein and

carbohydrate intake (Table 5). Rats received daily injections of either 2.0 mg DHTP, .2 mg DHTP, or the sesame oil vehicle. All injections were given in .1 ml sesame oil.

TABLE 5

BODY WEIGHT AND DIETARY INTAKE FOR RATS FED DIETS A AND B PRIOR TO DHTP OR OIL INJECTION
(Mean ± S.E.M.)

| GROUP | BODY WEIGHT (grams) | TOTAL FOOD (kcal/day) | PROTEIN (kcal/day) | CARBOHY-DRATE (kcal/day) |
|---|---|---|---|---|
| OIL | 355.6 ± 10.9 | 91.9 ± 3.9 | 23.2 ± 1.9 | 33.8 ± 1.1 |
| 0.2 mg DHTP | 354.6 ± 11.6 | 89.7 ± 5.2 | 21.2 ± 2.0 | 34.5 ± 1.9 |
| 2.0 mg DHTP | 355.2 ± 8.8 | 93.0 ± 3.0 | 22.0 ± 1.6 | 35.7 ± 2.0 |

Data analysis. Data were analyzed by one-way analyses of variance, followed by Newman-Keuls post hoc tests where appropriate.

Carcass analysis. Carcass composition was determined as in Experiment V except for the following modifications: Carcasses were baked for 3 days, ground in a Hamilton Beach food processor (#707), and returned to the oven and baked as in Experiment V. This modification shortened total baking time by about 1 week.

Lipid was extracted with 3 x 10 ml petroleum ether.

## Results

Total food intake. Hormone treatment significantly increased total caloric intake, $F$ (2,27) = 6.23, $p<.006$. Both groups of DHTP-treated animals consumed significantly more food over the 5-week injection period than did controls ($p<.01$, both groups, Figure 8). The two DHTP-treatment groups did not differ on this measure.

Protein intake. Protein consumption was also increased by DHTP, $F$ (2,27) = 5.12, $p<.01$. The two hormone-treated groups did not differ from one another, but both the .2 mg DHTP and 2.0 mg DHTP groups ate more calories as protein than did the oil controls ($p<.05$, both groups; Figure 8).

Carbohydrate intake. Carbohydrate intake was not significantly affected by DHTP, $F$ (2,27) = 2.6, $p$ = .09 (Figure 8).

Fat intake. Calories consumed as fat accounted for 37.9% of the total calories eaten, and thus were significantly higher for the two DHTP groups.

Body weight. Body weight gain was significantly

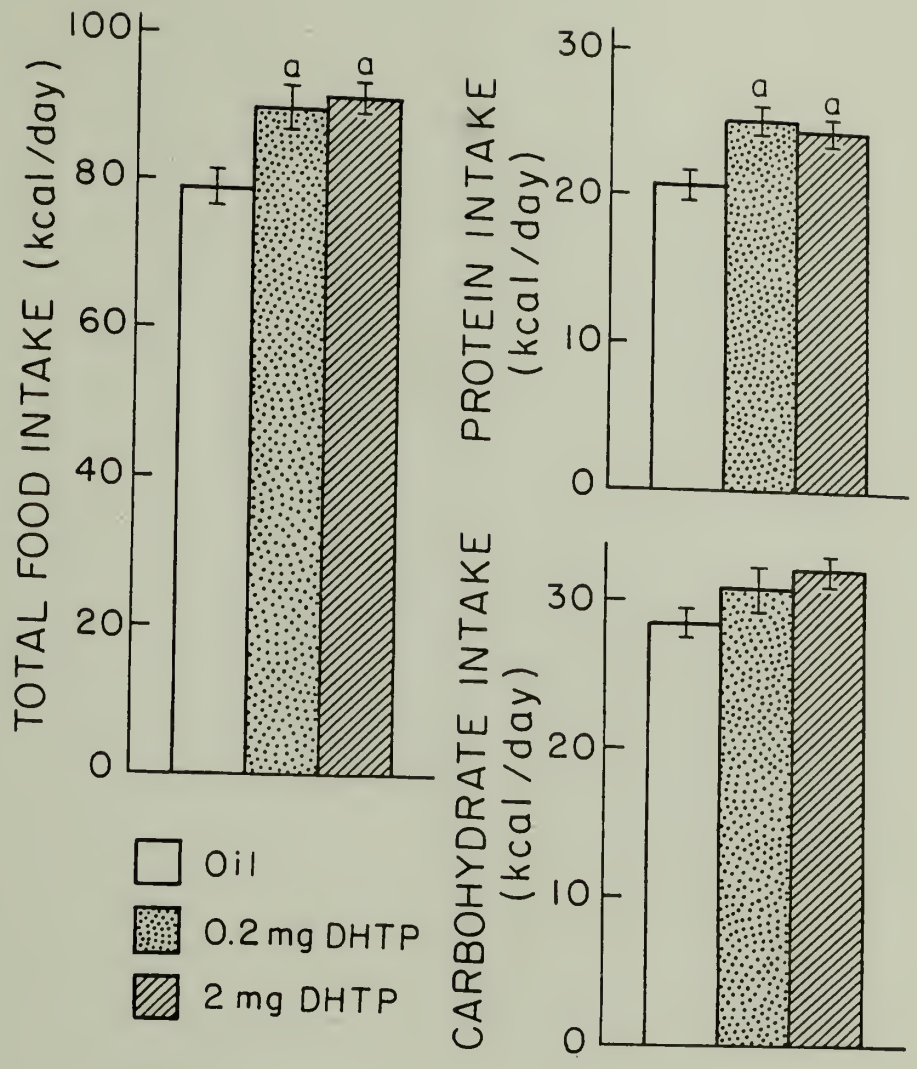



Fig. 8. Effects of DHTP or injection vehicle on total food (left panel), protein (upper right) and carbohydrate (lower right) intake in self-selecting GDX rats. Rats were given access to two isocaloric diets (Diets A and B) that differed only in protein and carbohydrate content. Values are means ± S.E.M.

[a]Significantly different from oil-injected group.

increased by DHTP treatment, $F$ (2,27) = 11.42, $p<.0003$. The groups receiving DHTP injections had gained significantly more weight at the end of the 5-week treatment than did the vehicle-injected group ($p<.01$, both groups), but total weight gain was not significantly different between the two DHTP groups (Figure 9). At the end of the injection period, mean body weights ($\pm$S.E.M.) for the oil, low DHTP and high DHTP groups were, respectively, 412.4 $\pm$ 13.4 g, 448.0 $\pm$ 17.3 g and 435.3 $\pm$ 12.3 g.

Carcass composition. There were no significant differences in the percentages of carcass water, $F$ (2,27) = .28, $p$ = .76; lipid, $F$ (2,27) = .03, $p$ = .97; or protein, $F$ (2,27) = .58, $p$ = .57, among the three groups (Figure 10).

## Discussion

The results of this experiment are in agreement with the report by Gentry and Wade (1976a) that DHTP increases body weight gain and food intake in GDX male rats. Rats given either a high or low dose of DHTP increase intake of all dietary components, but only protein consumption is significantly greater in these animals compared with the oil-treated control group.

Analysis of carcass composition reveals that the

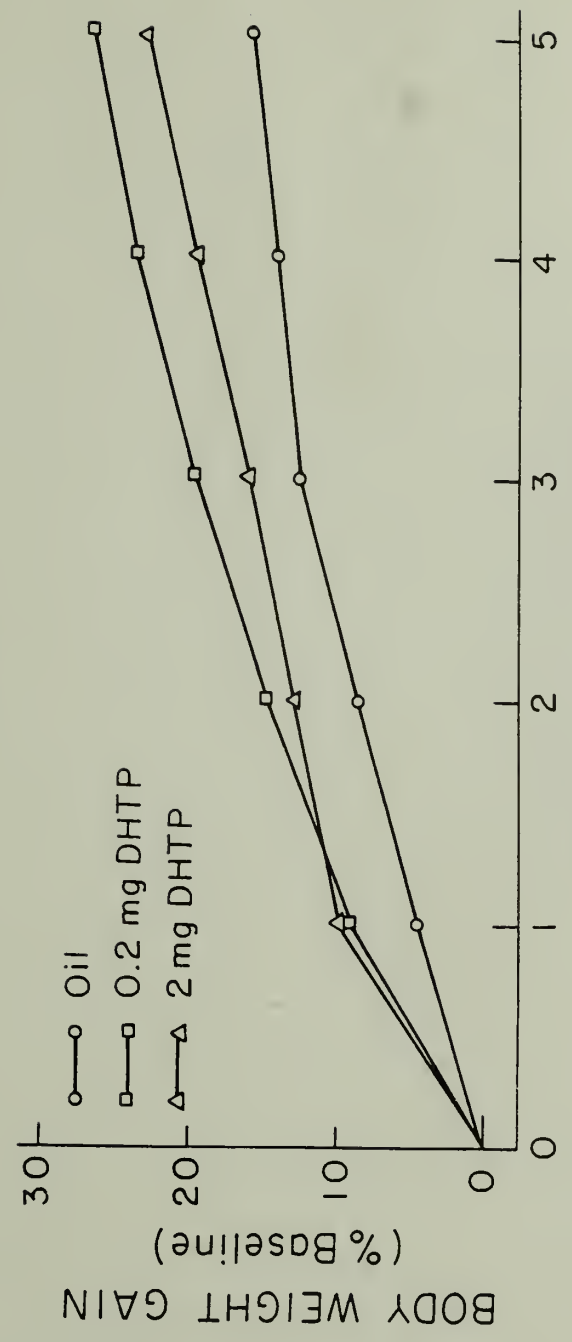



Fig. 9. Body weight gains of GDX, self-selecting rats receiving long-term DHTP or oil treatment.

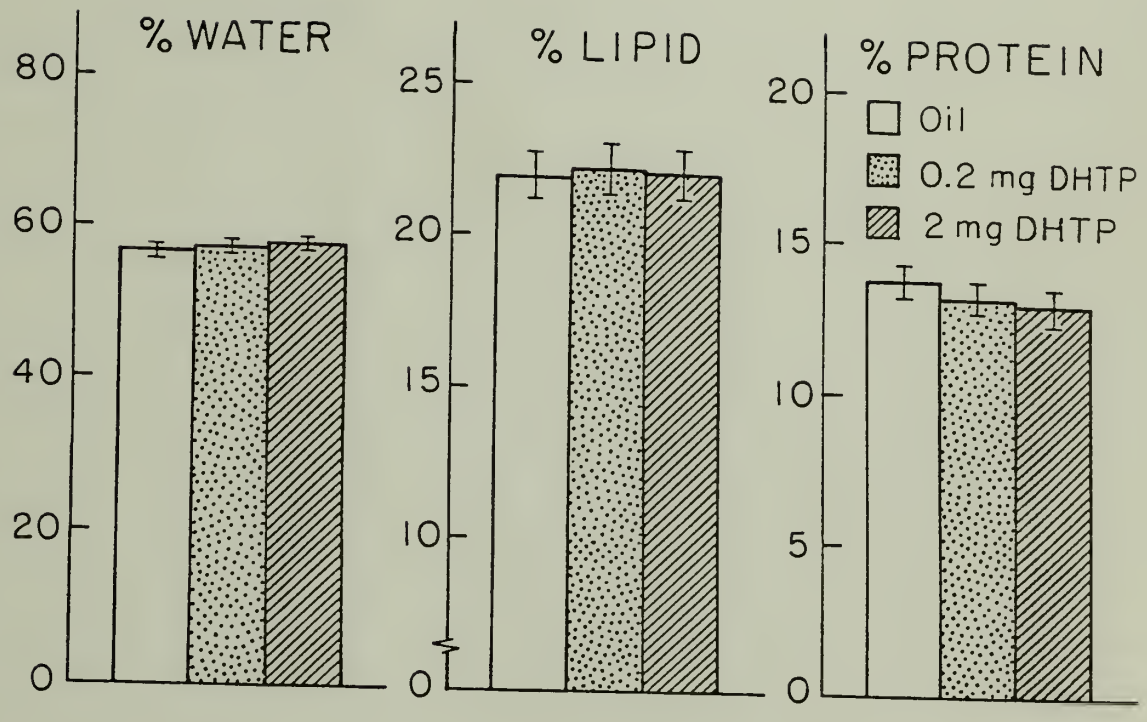



Fig. 10. Carcass composition of GDX rats given long-term DHTP or oil treatment. Data are presented as means ± S.E.M.

DHTP-induced weight gain is not restricted to one body component; the percentages of carcass water, protein, and lipid do not differ from those of controls. Although protein intake is increased by DHTP, the absolute amount of carcass protein is not affected by DHTP. That is, even though DHTP-treated rats weigh more than controls, there are no significant differences in the mean number of grams of protein in the carcasses of rats receiving either dose of DHTP compared to the oil-treated controls, $F$ (2,27) = .25, $p$ = .78 (mean amounts of protein (g) $\pm$ S.E.M. in carcasses of the oil, low DHTP and high DHTP groups are, respectively, 54.2 $\pm$ 1.8, 56.2 $\pm$ 2.9 and 53.9 $\pm$ 2.6).

## General Discussion

The results of these experiments show that both TP and DHTP stimulate food intake, protein intake and body weight gain in GDX male rats. However, several differences can be discerned in the actions of the two androgens on these measures, and a comparison of these actions may be instructive.

First, TP is more effective than DHTP in stimulating caloric intake and weight gain. After 27 days of injections, the .2 mg TP group had gained 16.5% more weight than did its oil-control group,

whereas after 28 treatment days the .2 mg DHTP group had gained only 9.2% more than its appropriate control group. Treatment with TP, but not DHTP also increased carcass protein content. Similarly, during the 27 days of treatment, the .2 mg TP group consumed an average of 15.8 kcal/day more than did the oil group, but the .2 mg DHTP group ate only 10.3 kcal/day more than the controls. These results confirm and extend the work of Gentry and Wade (1976a) and indicate that the actions of testosterone on eating and body weight are probably not mediated by the metabolite, 5α-dihydrotestosterone. This conclusion is consistent with the findings that muscle and kidney, where testosterone has substantial anabolic effects, have little or no 5α-reductase activity (Bardin et al., 1978; Krieg and Voigt, 1977; Mainwaring, 1977).

Second, treatment with TP increased both protein intake and carcass protein content, whereas DHTP increased protein intake without affecting carcass protein content. These findings indicate that, at least to some extent, the increased protein intake of androgen-treated rats is independent of peripheral anabolic actions of the hormones. However, the fact that TP was more effective than DHTP in stimulating protein intake raises the possibility that these

peripheral anabolic actions could contribute to TP-induced protein consumption.

Third, the two DHTP doses were equally effective in altering eating and body weight, but the two TP doses were not. Rats treated with the high dose of TP weighed and ate less than those treated with the low dose, confirming earlier findings. These relative decreases in food intake and body weight are due to reductions in carbohydrate intake and body fat content, respectively. These two findings are fully consistent with the hypothesis that intake- and weight-depressing effects of high TP doses are mediated by aromatized metabolites (i.e., estrogens). Treatment of OVX female rats with estradiol reduces body fat content (Gray and Wade, 1981), just as the high TP dose did in GDX males. Furthermore, Wurtman and Baum (1980) have recently shown that estradiol treatment decreases carbohydrate, but not protein, intake in rats given a protein-carbohydrate choice similar to that in Experiments V and VII. Taken together with the earlier finding that the intake- and weight-reducing effects of high doses of TP are prevented by concurrent administration of an aromatase inhibitor (Experiment I), the present results provide strong support for the

contention that aromatization plays a significant role in the actions of testosterone on eating and body weight.

# CHAPTER V

## COPULATION-INDUCED CHANGES IN BODY WEIGHT

Experiments I through VII were designed to investigate the effects of androgens on weight-related parameters in castrated male rats. In the majority of these studies rats were given one of two concentrations of an androgen and, for the aromatizable androgen, testosterone, the influence of the hormone on several variables was found to be differentiated on the basis of dose. This raises the question of whether any of the dose-dependent (particularly high-dose) effects of testosterone observed in the preceding experiments were the result of pharmacological manipulations of the testicular steroid, or whether similar changes are observed in intact male rats.

### Experiment VIII: Dietary Self-Selection, Body Weight and LPL Activity in Copulating Male Rats

There is evidence that intact male rats do experience changes in gonadal hormone levels under certain conditions. For example, testosterone levels rise after copulatory activity (Kamel, Mock, Wright and Frankel, 1975) or when male rats are exposed to stimuli associated with sexual encounters (Graham and Desjardins, 1980). When adult male rats are housed with intact

female rats or given repeated mating tests, body weight gain and carcass fat content decrease (Drori and Folman, 1964; Folman and Drori, 1969; Weizenbaum, 1978; 1981). Similar changes in body weight (Experiments I, V and VI; Gentry and Wade, 1976a) and adiposity (Hervey and Hutchinson, 1972; Kochakian, 1975; Kochakian and Webster, 1958) are observed in adult males given high doses of testosterone. This suggests that the changes in body weight and carcass composition seen in sexually active male rats may be the result of the copulation-induced increase in circulating testosterone. In addition, a small reduction in caloric intake has been observed in some sexually active male rats fed standard laboratory chow (Weizenbaum, 1981), and food intake is lower in GDX rats given high doses of TP than it is in similar rats receiving lower doses of TP (Experiment I, Gentry and Wade, 1976a).

The purpose of Experiment VIII was to investigate the effects of copulation on behavioral and physiological end-points that are sensitive to testosterone. Sexually active and sexually inactive male rats were given access to a pair of isocaloric diets that were equal in fat, but contained differing amounts of protein and carbohydrate. (The diets in this experiment were the same as those used to establish the effects of TP

(Experiment V) and DHTP (Experiment VII) on intake of dietary components.) Body weight as measured throughout the experiment, and at the completion of testing, adipose tissue LPL activity and seminal vesicle weights were measured. Seminal vesicle weight has been shown to be increased in mated male rats compared to unmated control rats, and this increase is likely due to elevated testosterone levels (Folman and Drori, 1969).

## Method

Animals and housing. Thirty adult CD-strain rats (Charles River Breeding Laboratories), weighing 350-450 g at the beginning of the experiment, were housed individually under a 12:12 hour light-dark cycle (lights off at 1200 hr). Purina rodent chow pellets were available until the introduction of the experimental diets; tap water was available throughout the experiment.

Stimulus animals for all mating tests were OVX females treated with EB and progesterone to induce estrous behavior. Males used in the experiment were those showing male copulatory behavior in at least one of two pretests conducted 2-3 weeks before the introduction of the experimental diets.

Diets. Diets were the low protein (Diet A) and high protein (Diet B) diets used in Experiments V and VII. Rats were given two weeks to adapt to the experimental diets prior to data collection. At the end of the two-week adaptation period, all animals were eating from both food jars. The position of each jar was varied throughout the adaptation and experimental periods.

Procedure. After adaptation to the diets, food intake and body weight were measured twice weekly between 0900 and 1000 hr. After one week of baseline data collection, rats were assigned to one of three groups (n = 10 each) matched for body weight, total caloric intake and consumption of the two diets. The treatment groups are described below.

Copulators (C). These animals were given access to receptive females two (Weeks 1-4) or three times (Weeks 5 and 6) per week. During these sex-behavior trials, each male was placed in a circular test arena (60 x 60 x 25 cm high) with a wood chip covered floor for 5 minutes of adaptation. Following this adaptation period, 2 or 3 receptive females were placed in the arena and remained there until the male ejaculated. Sex behavior trials were conducted during the first 4 hours of the dark phase of the illumination cycle, under dim red light. All males ejaculated on all

trials. The latencies to ejaculation decreased over the experimental period (15-45 minutes for Week 1; 3-20 minutes for Week 6).

Arena Controls (AC). These animals were individually placed in an empty test arena containing clean wood chips on the floor, and they remained in the arena for the duration of the sex-behavior trials of their counterparts from group C.

Home Cage Controls (HC). No manipulations were performed on this group. The animals were handled only to obtain body weights.

Several weeks into the experiment one animal in the AC group developed a habit of digging through his food and tossing it out of his cage. All data for this rat were discarded as were data from one rat in the HC group who one week later, also began spilling his food. Since the two rats had very similar body weights at the beginning of the experiment, data were also discarded from one animal in the C group whose baseline body weight was closely matched to the initial body weights of these two animals. The recalculated baseline data for the three experimental groups (n = 9 each) are given in Table 6.

Tissue preparation and LPL assay. All animals were killed by decapitation between 0900 and 1000 hr, 24 hours

TABLE 6

BASELINE BODY WEIGHT AND INTAKE OF DIETARY COMPONENTS IN SELF-SELECTING INTACT MALE RATS (Mean ± S.E.M.)

| GROUP | BODY WEIGHT (grams) | TOTAL FOOD (kcal/day) | PROTEIN (kcal/day) | CARBOHY-DRATE (kcal/day) |
|---|---|---|---|---|
| COPULATOR | 417.8 ± 10.3 | 99.7 ± 3.1 | 21.5 ± 2.1 | 40.4 ± 2.7 |
| ARENA | 415.7 ± 11.4 | 98.3 ± 3.1 | 20.3 ± 2.1 | 40.9 ± 1.7 |
| HOME CAGE | 414.0 ± 12.1 | 99.7 ± 4.6 | 24.7 ± 1.5 | 37.2 ± 3.5 |

after the last sex-behavior trial. Epididymal adipose tissues were rapidly dissected and assayed for LPL activity as described in Experiment II. Seminal vesicles were dissected, cleaned and weighed wet. Dry weights of the seminal vesicles were obtained after the tissues had been dried in an oven at 35°C for 24 hours.

Data analysis. All food intake data were converted to calories. Separate analyses were performed on total caloric, protein and carbohydrate intake, as well as on body weight gain (% baseline) over the 6-week experimental period. Comparisons across groups were made using one-way analyses of variance. Significant $F$ values ($p<.05$) were followed by Newman-Keuls post

hoc tests.

## Results

Baseline body weights did not differ significantly across groups, $F$ (2,24) = .03, $p$>.90. However, by the end of the six-week experimental period the groups differed significantly on mean body weight gain, $F$ (2,24) = 14.5, $p$<.01. Individual comparisons of group means showed that the C group had gained significantly less weight than had the two control groups ($p$<.01, both tests; Figure 11, Table 7). Weight gains of the AC and HC groups were not significantly different.

TABLE 7

EFFECTS OF COPULATION ON BODY WEIGHT AND FOOD, PROTEIN AND CARBOHYDRATE INTAKE (Mean ± S.E.M.)

| GROUP | BODY WEIGHT (grams) | TOTAL FOOD (kcal/day) | PROTEIN (kcal/day) | CARBOHYDRATE (kcal/day) |
|---|---|---|---|---|
| COPULATOR | 471.8 ± 14.4 | 89.1 ± 3.1 | 21.3 ± 1.1 | 34.3 ± 2.0 |
| ARENA | 499.1 ± 14.0 | 91.0 ± 2.9 | 21.0 ± 1.6 | 35.6 ± 1.1 |
| HOME CAGE | 505.4 ± 15.2 | 97.5 ± 3.0 | 25.4 ± 1.5 | 34.6 ± 1.1 |

Analyses of variance indicated no significant differences on any of the baseline food intake measures (Table 6). Table 7 shows food intake data averaged over

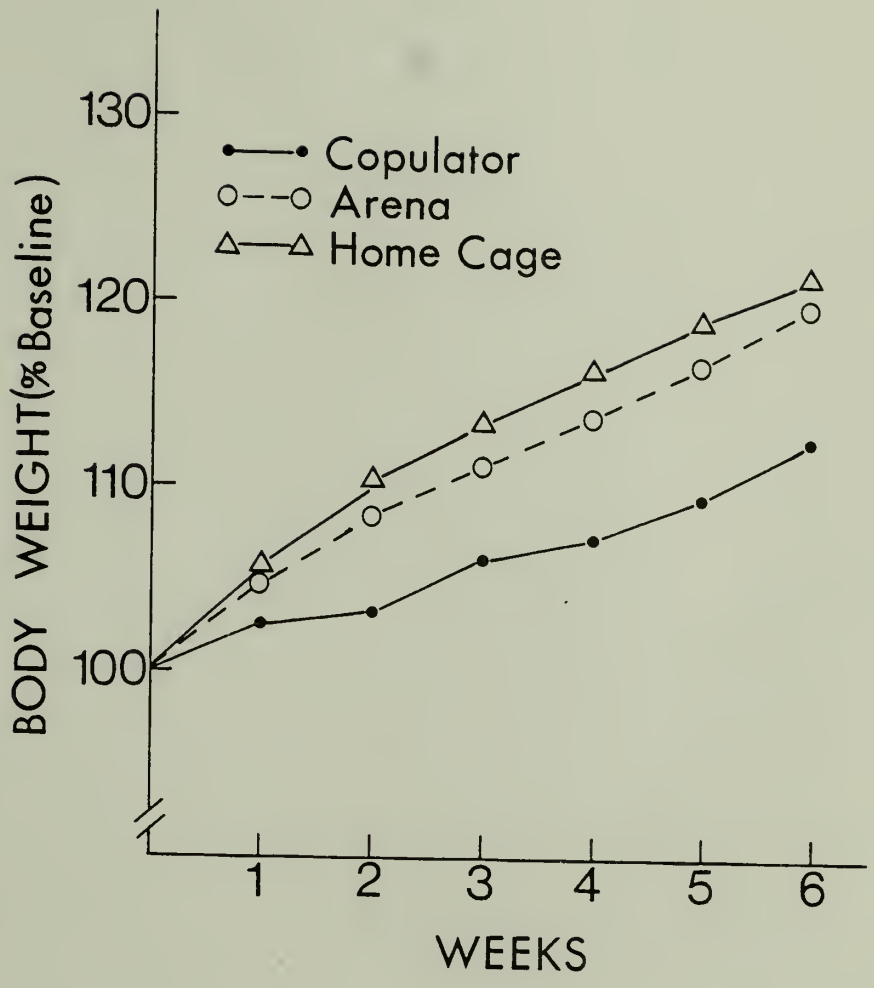



Fig. 11. Effect of copulation on body weight gain. Male rats were either allowed to copulate to ejaculation two (Weeks 1-4) or three (Weeks 5 and 6) times per week (Copulator), given access to an empty testing arena (Arena) or left undisturbed in their cages (Home Cage). Rats had access to Diets A and B.

the experimental period for the three groups. The groups did not differ significantly on total caloric intake, $F$ (2,24) = 2.12, $p$>.14; protein intake, F(2,24) = 3.11, $p$>.06; or carbohydrate intake, $F$ (2,24) = .23, $p$>.70. It should be noted that although the three treatment groups did not differ significantly on amount of protein consumed, the $p$ value was close to statistical significance. Thus, to ensure that protein intake was not significantly elevated in the HC group, data were converted to percent baseline for further analysis. Again, no significant differences were found across groups, $F$ (2,24) = .03, $p$>.90 (mean intakes of protein (% baseline) $\pm$ S.E.M. for C, AC and HC groups were, respectively, 104.2 $\pm$ 6.6, 106.4 $\pm$ 5.4 and 106.7 $\pm$ 10.7. Similarly, no significant differences were found across groups for percent change from baseline intake on the two other intake measures.

Individual $t$-tests indicated that the AC and HC groups did not differ significantly in terms of either wet or dry seminal vesicle weights relative to total body weights, so the two groups were combined on each of these measures for further statistical analyses. Animals in group C had significantly heavier mean seminal vesicle wet weights relative to total body weight, $t$ = 2.14, $p$<.05, and heavier dry vesicle

weights relative to total body weight, $t$ = 2.30, $p$<.02 than the other groups combined (Table 8).

Epididymal adipose tissue LPL activity was not changed as a result of copulation, $F$ (2,24) = .06, $p$>.90.

TABLE 8

EFFECTS OF COPULATION ON ADIPOSE TISSUE LPL ACTIVITY AND SEMINAL VESICLE WEIGHT
(Mean ± S.E.M.)

| GROUP | LPL ACTIVITY* | SEMINAL VESICLE WEIGHT | |
|---|---|---|---|
| | | WET WEIGHT (mg) /BODY WEIGHT (g) | DRY WEIGHT (mg) /BODY WEIGHT (g) |
| COPULATOR | .095 ± .02 | 2.4 ± .15 | .74 ± .05 |
| ARENA | .102 ± .02 | 2.0 ± .11 | .62 ± .03 |
| HOME CAGE | .097 ± .01 | 2.1 ± .13 | .64 ± .05 |

*LPL activity is expressed as μ mole free fatty acid released per hour per mg protein.

## Discussion

Sexual activity produced a marked reduction in body weight gain, thus confirming earlier observations (Drori and Folman, 1964; Weizenbaum, 1978; 1981). Previous research has shown that this effect on body weight is specific to copulation; access to non-receptive females, voluntary exercise, or exposure to a novel cage fail to

mimic the effects of copulation (Weizenbaum, 1981). In the present experiment, experience with the testing arena was clearly ineffective in reducing body weight. However, it cannot be determined from the available data whether the reduction in body weight results from actual copulation or from exposure to receptive females. There is evidence that neuroendocrine changes in male rats can be induced by previously neutral stimuli that become associated with access to receptive females (Graham and Desjardins, 1980).

The decreased rates of body weight gain seen in copulating animals are similar to the body weight changes of castrated rats treated with high doses of TP (Experiments I, V and VI; Gentry and Wade, 1976a). Furthermore, seminal vesicle weights were elevated in rats allowed to copulate, suggesting that endogenous testosterone levels were increased in these animals compared to unmated control rats.

When high doses of TP are given to GDX male rats, the subsequent reduction in body weight gain is generally accompanied by a reduction in food intake (Experiments I, V and VI). In contrast, copulation-induced changes in body weight were not associated with hypophagia. Other reports have shown that sexual activity can reduce food intake (primarily in young male rats)

but that this effect is not necessary for weight reduction (Weizenbaum, 1981). Copulation did not significantly affect the intake of protein or carbohydrate. This is in contrast to the findings in Experiment V in which daily injections of 2 mg TP decreased carbohydrate intake in castrated male rats compared to carbohydrate intake of castrated rats receiving injections of .2 mg TP. In that experiment, both doses of TP increased protein intake relative to rats treated with the injection vehicle alone.

Adipose tissue LPL activity is reduced by doses of TP that decrease food intake, body weight gain (Experiments I and II) and carcass fat content in GDX rats. Although the sexually active males gained significantly less weight than rats in either control group, adipose tissue LPL activity was unchanged in the mated animals. These data suggest that copulation-induced decreases in adiposity (Weizenbaum, 1981) do not require changes in adipose tissue LPL activity. Changes in adiposity without alterations in activity of this enzyme have been observed in female rats (Gray and Wade, 1981).

In summary, although both copulation and testosterone treatment may decrease body weight gain and adiposity, the mechanisms by which they cause such

changes may differ. Whereas high doses of TP may cause reductions in these measures in part by reducing LPL activity, copulation-induced changes in weight gain and carcass fat do not appear to require changes in adipose tissue LPL activity. There is no evidence, however, that the reduction in LPL activity in castrated males treated with high doses of TP is the sole factor causing decreased weight gain and adiposity; thus, it is possible that other mechanisms (perhaps similar to those involved in copulation-induced changes in body weight) are involved. It is interesting to note that the levels of circulating testosterone in mated male rats are dependent upon the frequency of copulation, such that testosterone levels rise as the frequency of mating increases (Folman and Drori, 1969). Folman and Drori (1969) found that male rats allowed to copulate three times per week to exhaustion had less body fat than males copulating once a week to exhaustion; this latter group had fewer grams of carcass fat than males allowed to copulate once a week to two ejaculations. The carcass fat contents of the rats with the lowest frequency of mating did not differ significantly from those of unmated control rats. These data support the contention that high levels of circulating testosterone decrease carcass adiposity. The

changes in body composition that occur after prolonged periods of cohabitation with females (Drori and Folman, 1964) may, in part, be mediated by sustained increased levels of endogenous testosterone similar to those produced by long-term treatment with high doses of TP.

Although other mechanisms by which copulation may decrease body weight and adiposity are unknown, it is possible that they might include actions of adrenal hormones. For example, glucocorticoids promote mobilization of depot fat and inhibit lipogenesis (Martin, 1976). That glucocorticoid titers may be enhanced in mated males is suggested by the observation that adrenal weights increase with increasing frequency of mating in male rats (Folman and Drori, 1969). The role of the adrenals (if any) in copulation-induced weight loss awaits further testing.

# CHAPTER VI

# INVESTIGATION OF PROGESTERONE-INDUCED WEIGHT GAIN IN MALE RATS

## Experiment IX: Effects of Progesterone on Body Weight, Food Intake and Carcass Composition in EB- or TP-Treated, GDX Rats

Estradiol decreases food intake, body weight and adiposity in OVX and intact female rats. Progesterone alone has no effect on any of these measures. However, when given in conjunction with estradiol, body weight, eating and adiposity increase (see Chapter I for references). As discussed earlier, some of the changes in adiposity seen after treatment with EB or EB plus progesterone may be mediated by changes in adipose tissue LPL activity (Wade and Gray, 1979). The effects of these gonadal steroids on adipose tissue LPL activity are likely mediated via cytoplasmic estrogen and progestin receptors in female rats (Gray and Wade, 1979; Wade and Gray, 1978).

In male rats given high doses of TP, the observed decreases in food intake, body weight and adiposity (Chapters II and IV) are likely mediated by estrogenic products of TP. Progesterone given concurrently with high doses of TP or EB antagonizes the weight-reducing effects of high doses of TP or EB in GDX males, although

such treatments do not alter food intake (Gentry and Wade, 1976a; 1976b).

As in female rats, EB treatment depletes cytoplasmic estrogen receptors in adipose tissues and decreases adipose tissue LPL activity in male rats (Gray and Wade, 1980). However, unlike its effect in female rats, EB does not induce progestin receptors in adipose tissues in male rats, and progesterone does not affect LPL activity in EB-primed males (Gray and Wade, 1980). The question is therefore raised as to how progesterone acts to increase body weight in castrated male rats given either estradiol or repeated injections of high doses of testosterone.

The purpose of Experiment IX was to investigate the effects of progesterone on body weight gain and carcass composition in GDX male rats receiving concurrent injections of EB or high doses of TP. Food intake was also measured.

Method.

Animals and housing. Forty-four adult CD-strain rats (Charles River Breeding Laboratories) were housed as in Experiment VIII. Rats were given free access to Purina rodent chow and tap water, and were castrated as previously described.

Procedure. One month after castration, animals

each received a daily injection of .1 ml sesame oil for 1 week and baseline food intake and body weight were recorded. Rats were then matched according to food intake and body weight and assigned to one of the five following groups: Oil: .2 ml sesame oil/day; EB: 2 µg EB/day; EB + P: 2 µg EB/day + 5 mg progesterone/day; TP: 2.0 mg TP/day; TP + P: 2.0 mg TP/day + 5 mg progesterone/day (n = 8 for Oil group, n = 9 all other groups; Table 9). Hormones were dissolved in sesame oil and injections were given such that each rat received a total volume of .2 ml daily. Food intake (including spillage) and body weight were measured twice weekly.

Carcass analysis. At the end of the experimental period animals were killed with an overdose of Nembutal and prepared for carcass analyses as described in Experiment V. Carcass analyses were performed as reported in Experiment V with the exception that lipid was extracted with 4 x 10 ml petroleum ether.

Data analysis. Data for the week preceding initiation of hormone injections were used as baseline. Food intake and body weight data were analyzed as described in Experiment I except that data were divided into two 4-week blocks for analysis.

Results.

Food intake (Figure 12, Table 9). During the first four weeks of treatment, food intake was lowest for the group receiving EB, although the groups did not differ significantly in amount of food eaten, $F$ (4,39) = 2.44, $p$ = .06. Total caloric intake differed across groups during the final four weeks of hormone treatment, $F$ (4,39) = 3.57, $p<.02$. The EB-induced hypophagia that was evident during Weeks 1-4 was no longer observed at Weeks 4-8; caloric intake of the EB treatment group did not differ significantly from that of the oil-treated control group. Food intake of the group receiving TP did not differ significantly from either the EB or Oil group. Whereas food intake was significantly increased in the TP + P group compared to the Oil- or EB-treated rats ($p<.05$, $p<.01$, respectively), food intake was not significantly increased relative to the TP group. Although progesterone treatment also increased food intake in EB-primed rats during the last month of hormone treatment, this increase was not significant (compared with either the EB or Oil group). In fact, caloric intake of the EB + P group did not differ significantly from that of any other group.

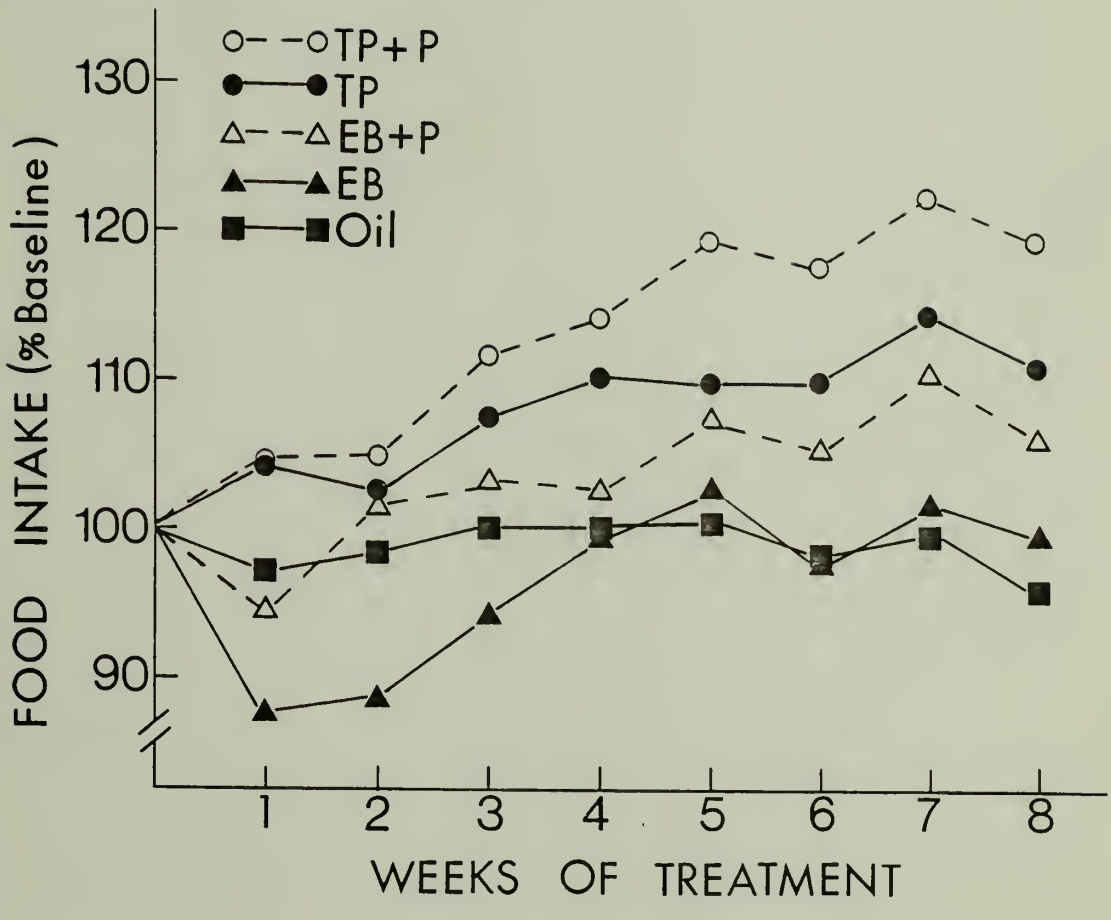



Fig. 12. Effects of EB or a high dose of TP given alone or given concurrently with progesterone on food intake in GDX rats.

TABLE 9

EFFECTS OF PROGESTERONE (P) ON BODY WEIGHT AND FOOD INTAKE IN EB- OR TP-TREATED RATS (Mean ± S.E.M.)

| GROUP | BODY WEIGHT (g) | | | FOOD INTAKE (kcal/day) | | |
|---|---|---|---|---|---|---|
| | BASELINE | WEEK 4 | WEEK 8 | BASELINE | WEEKS 1-4 | WEEKS 5-8 |
| OIL | 414.0 ±11.3 | 464.1 ±11.6 | 499.5 ±11.8 | 96.9 ±2.8 | 95.7 ±2.2 | 95.2 ±1.5 |
| EB | 412.9 ±10.6 | 418.1 ±11.8 | 439.4 ±12.6 | 98.8 ±4.2 | 91.0 ±3.2 | 99.3 ±4.8 |
| EB + P | 414.6 ±10.7 | 438.2 ±10.7 | 463.6 ±11.7 | 94.5 ±3.7 | 94.4 ±2.7 | 101.1 ±3.6 |
| TP | 414.0 ±11.6 | 473.1 ±13.3 | 495.6 ±13.2 | 94.3 ±3.1 | 99.4 ±2.8 | 104.5 ±2.4 |
| TP + P | 414.3 ±10.8 | 476.7 ±13.9 | 512.0 ±15.9 | 94.0 ±1.9 | 102.1 ±2.8 | 112.8 ±3.8 |

Body weight (Figure 13, Table 9). After four weeks of injections the treatment groups differed significantly in body weight gain, $F$ (4,39) = 43.41, $p<.00001$. Because groups found to be significantly different (Newman-Keuls tests) at four weeks were the same groups that differed significantly at eight weeks, the results of post hoc comparisons of means at eight weeks will be given.

Unlike the transient effect of EB on food intake, EB produced a lasting decrease in body weight. At the

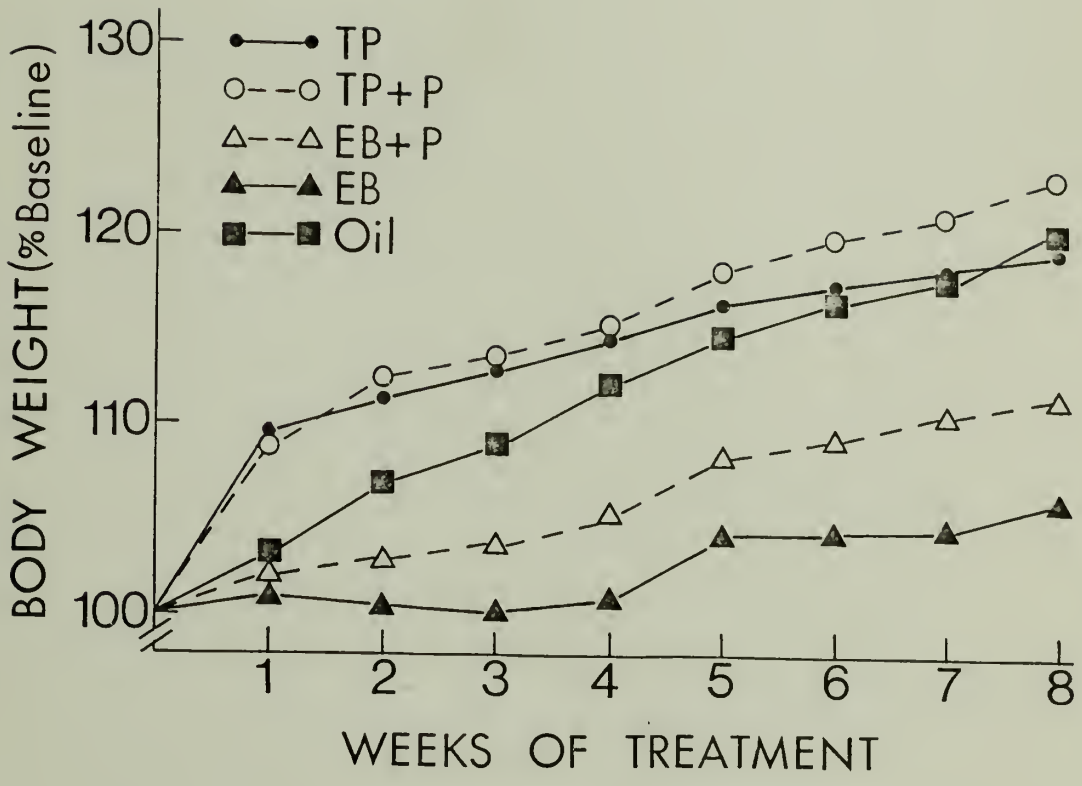



Fig. 13. Effects of 2 µg EB; 2 µg EB + 5 mg P; 2 mg TP + 5 mg P; or oil on body weight in GDX rats.

completion of the 8-week injection period, the EB-treated group had gained significantly less weight than either the oil controls or the two TP-treated groups, $F$ (4,39) = 24.21; $p<.00001$ ($p<.01$, all post hoc tests). The EB-induced reduction in weight gain was attenuated by progesterone treatment ($p<.05$). However, the weight gain of the EB + P group was still significantly less than that of the vehicle-treated animals ($p<.01$; and it was less than that of either TP treatment group, $p<.01$, both tests). Mean body weight gain of the TP treatment group did not differ significantly from that of the Oil treatment group. In agreement with the food intake data, progesterone (which did not significantly increase caloric intake relative to TP treatment alone) did not significantly increase body weight gain in TP-treated males. In contrast to the food intake results, TP + P treatment (which significantly increased food intake relative to treatment with the injection vehicle alone) did not significantly increase body weight compared to Oil-treated controls.

Carcass composition (Figure 14). No significant differences were found on the percentages of carcass lipid, protein or water across groups. However, rats in the TP + P group had significantly more grams of protein in their carcasses (mean + S.E.M. = 109.5 $\pm$

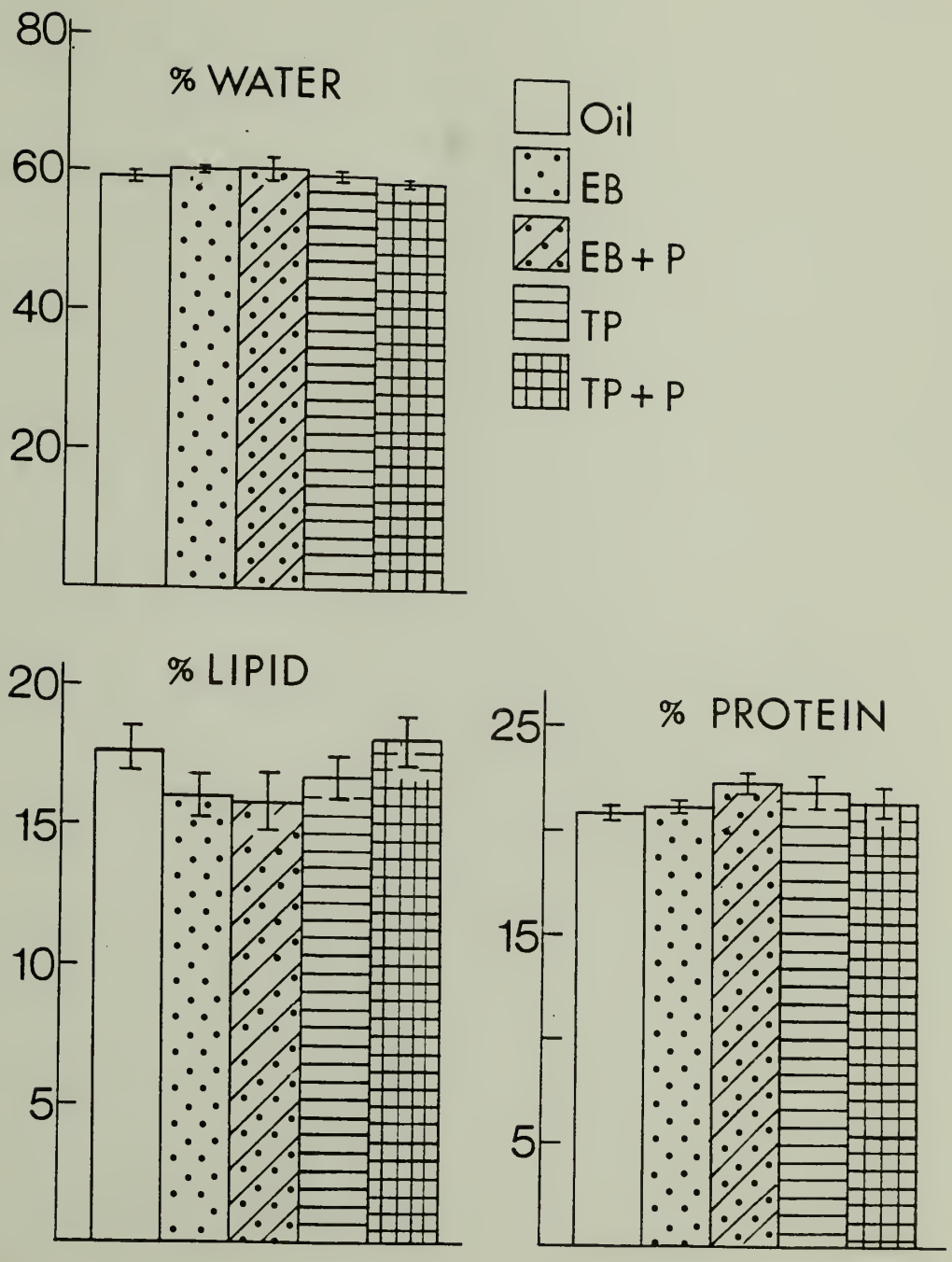



Fig. 14. Carcass composition of GDX rats given long-term treatment with either EB or a high dose of TP alone, or EB + P, TP + P, or oil injection vehicle.

4.3 g) than did rats in the EB (93.4 ± 3.8 g) group, $F$ (4,37) = 2.61, $p<.05$ ($p<.05$, Newman-Keuls). No other significant differences were found on absolute amount of carcass protein (mean ± S.E.M., Oil: 102.7 ± 2.5 g; EB + P: 104.5 ± 3.5 g; TP: 106.5 ± 4.5 g). The TP + P group also had significantly more (absolute) carcass lipid (93.1 ± 6.4 g) than did the EB group (70.0 ± 5.0 g), $F$ (4,37) = 2.84, $p<.04$ ($p<.05$, Newman-Keuls). No other significant differences were found between groups (Oil = 87.6 ± 6.7 g lipid; EB + P = 73.3 ± 5.8 g; TP = 83.1 ± 5.2 g).

## Experiment X: Effects of TP on Induction of Cytoplasmic Progestin Receptors in Epididymal Adipose Tissue in ADX, GDX Rats

As stated in Experiment IX, progesterone increases body weight in GDX male rats given EB or high doses of TP (Gentry and Wade, 1976a; 1976b). However, EB does not induce progestin receptors in adipose tissues in male rats. The increased weights of OVX, EB-treated female rats given progesterone are due primarily to increased carcass fat contents.

Experiment X was designed to determine whether a high dose of TP can induce progestin receptors in adipose tissue in male rats. Experiment IX revealed that the weight gains of TP-primed, progesterone

treated males are, in part, due to increased fat content (although the increased fat content of the TP + P group was not statistically significant). The present experiment was conducted prior to the completion of Experiment IX, at which time it was expected that progesterone treatment would increase carcass fat in rats treated with high doses of TP. Thus, at that time it was of interest to determine whether the increase was mediated via progestin receptors in adipose tissues.

Method.

Animals and housing. Twenty-seven CD-strain rats were housed individually, and maintained on a 12 hour light:12 hour dark cycle (lights on 0600 hr). Animals were orchiectomized through a midscrotal incision and adrenalectomized (ADX) via bilateral dorsolateral incisions. All surgery was done under methoxyflurane anesthesia. Rats were maintained on Purina rodent chow and 0.9% saline and libitum.

Procedure. One day after surgery, rats were matched by body weight and divided into three groups (n = 9 each). Rats each received a daily injection of either 2 µg EB or 2 mg TP in .1 ml sesame oil, or .1 ml of the injection vehicle alone.

Cytoplasmic progestin receptor assay. After 10 days of injections, rats were given an overdose of sodium pentobarbital. Epididymal adipose tissues were rapidly dissected and weighed to the nearest 0.1 mg. Cytoplasmic progestin binding was assayed according to Gay and Wade's modification of the method of Maclusky and McEwen (1978).

Results. Neither EB nor TP treatment induced cytoplasmic binding sites for the synthetic progestin, 17α, 21-dimethyl-19-norpregna-4,9-diene-3,20-dione (R 5020), in epididymal fat in ADX, GDX male rats. The mean (± S.E.M.) amount of [$^{3}$H]R 5020 specifically bound for the EB, TP and Oil groups was, respectively, 1.9 ± .6 fmol/mg protein; 2.0 ± .6 fmol/mg protein, and 2.4 ± .9 fmol/mg protein. Differences between groups were not statistically significant, $F$ (2,25) = .20, $p$ = .82.

## Discussion

Many of the effects of the various hormone treatments on food intake and body weight gain in Experiment IX are in agreement with findings reported earlier by Gentry and Wade (1976a; 1976b). First, EB significantly decreased weight gain and produced a transient, statistically nonsignificant reduction in food intake

in GDX male rats as earlier reported (Gentry and Wade, 1976b). Second, as demonstrated previously (Gentry and Wade, 1976b), progesterone significantly attenuated EB-induced weight loss without affecting food intake. Third, daily injections of 2 mg TP initially increased rate of weight gain in GDX rats, but with repeated treatment weight gain slowed such that it did not differ from weight gain of oil-treated rats; whereas food intake of the TP group did not differ from that of control animals in any part of the injection period (as was found by Gentry and Wade, 1976a, but c.f. Experiments V and VI). In contrast, although weight gain was greatest in the TP + P group it did not differ significantly from that of either the TP or the oil group as it did in the Gentry and Wade study (1976a). In addition, food intake was significantly increased in the TP + P group relative to the oil treatment group whereas the food intakes of these two groups did not differ in the above report.

The lack of effect of TP + P on food intake (compared to oil treatment) reported by Gentry and Wade is rather surprising since in that study a 1 mg dose of TP was used. This dose of TP alone increased food intake compared to oil treatment in one experiment in that report, although this dose of TP alone apparently

did not significantly increase food intake in the experiment in which it was also given concurrently with progesterone. In Experiment IX, an even higher dose of TP (2 mg), one which reliably does not increase food intake compared to oil treatment, was used. That food intake was increased (compared to oil controls) when progesterone was given in conjunction with the 2 mg dose of TP is thus all the more striking.

In Experiment IX, as in other experiments, gonadal hormones sometimes affected body weight without affecting food intake. As discussed previously (Chapter I; see also Wade and Gray, 1979), there are data which show that changes in food intake that follow treatment with some steroid sex hormones are not always necessary and/or sufficient to cause the resultant changes in body weight. The present experiment offers an example in which food intake was increased without a concomitant change in body weight. Food intake of rats in the TP + P group during the last month of the experiment increased compared to that of vehicle-treated animals, whereas body weight gain did not differ significantly between the groups. Indeed, at the end of the experiment, the TP + P group was eating 18.5% more calories daily than was the control group, but weighed only 2.5% more than the rats in this group.

The failure to find a difference in body weight between the oil and TP + P groups may in part be due to the large weight gain of the oil-treated group. Weight gains of the oil treatment groups in Experiments V-VIII and the Oil + Cholesterol control group in Experiment I ranged from about 10-13% whereas the oil-treated rats in this experiment gained nearly 21% above baseline levels.

Estradiol benzoate decreased body weight compared to controls and analysis of carcass composition revealed that the weight loss was mainly due to loss of body lipid. Although progesterone treatment attenuated the weight-reducing effects of EB, no difference in carcass composition were found between the EB and EB + P groups. However, that the percentage of carcass protein was highest in the EB + P group is of interest in light of the suggestion by Gray and Wade (1980) that progesterone may have some anabolic actions in EB-treated male rats.

As in Experiments V and VI, repeated injections with high doses of TP did not increase weight gain above the level observed in control animals, and caused a slight reduction in carcass lipid content (which was significant in Experiments V and VI, but not in the present experiment). In rats given 2 mg doses of TP, progesterone also increased body weight slightly as it

did in EB-treated rats. The small increase in body weight was reflected by a slight increase in percent lipid in the TP + P group. As shown in Figure 14, progesterone treatment actually reversed the anorexic effects of high doses of TP on body lipid such that lipid content of the TP + P and Oil groups appeared quite similar. That many of the small changes in carcass content were not found to be statistically significant is likely a result of small sample size and high variability within groups. Further work is needed to verify whether the increased lipid content of progesterone-treated, TP-primed animals (compared to TP-treated rats) reflects an actual effect of progesterone or whether it is indeed due to chance.

In agreement with earlier findings, EB did not induce cytoplasmic progestin binding sites (Gray and Wade, 1980). Similarly, repeated injections of 2 mg TP were ineffective in inducing progestin binding sites. As reported earlier, it is likely that some of the effects of high doses of TP are mediated via estrogenic metabolites of the hormone. High doses of TP deplete cytoplasmic estrogen receptors and decrease LPL activity in adipose tissues; these effects are blocked by an aromatization inhibitor (Experiment I). That neither EB nor TP induce cytoplasmic progestin

binding sites in adipose tissue in male rats suggests that the small increases in body weight and carcass adiposity in the TP + P group are not mediated by progesterone binding in epididymal adipose tissues. Progesterone has no effect on LPL activity in EB-primed male rats (Gray and Wade, 1980). Since the effects of high doses of TP on LPL activity are probably due to estrogenic metabolites, it is likely that progesterone has no effect on LPL activity in GDX male rats given high doses of TP. There are recent data that show that treatments which fail to induce progestin binding sites in adipose tissues also fail to affect LPL activity when given concurrently with progesterone (Gray and Wade, 1980; 1981).

The mechanism by which TP + P acts to increase food intake is not known. As before, that TP failed to induce progestin binding sites in epididymal fat suggests that TP + P might not affect adipose tissue LPL activity. Gray and Wade (1981) report that the nonsteroidal antiestrogen, nafoxidine, antagonizes the induction of cytoplasmic progestin binding sites by EB in parametrial adipose tissue, and that it has no effect on adipose tissue LPL when given concurrently with progesterone. Yet, when nafoxidine is given concurrently with progesterone, food intake, body weight

and carcass lipid content increase. These data suggest that a change in adipose tissue LPL activity is not the sole mechanism by which progesterone acts to increase body weight and food intake. In the absence of any data to the contrary, it is possible that central mechanisms and/or other peripheral mechanisms are involved in increasing food intake in GDX male rats receiving long-term treatment with high doses of TP plus progesterone.

# CHAPTER VII

## ABSENCE OF DIETARY EFFECTS ON ANDROGEN-SENSITIVE, WEIGHT-RELATED PARAMETERS IN MALE RATS

### Experiment XI: Food Intake and Body Weight in Rats Fed Chow or High Fat Diet: Effects of Orchiectomy

Two recent studies have found that OVX rats fed a high fat diet are more responsive to the anorexic effects of EB than are OVX females fed a standard chow diet (Young, Nance and Gorski, 1978; Young, Nance, Gorski and Gordon, 1979). When OVX female rats fed a high fat or chow diet are each given a single injection of 6 µg EB, food intake declines significantly for four days post-injection. However, this decrease in food intake is significantly greater for rats fed a high fat diet. Although body weights of both groups decrease during the post-injection period, the declines in weights are similar for both groups (Young et al., 1978). The hyperresponsiveness of high fat diet fed females to EB may be specific to food intake since EB-induced mating behavior is similar in chow or high fat diet fed rats (Young, Nance, Gorski and Gordon, 1979). In GDX male rats fed a high fat diet, a single injection of 6 µg EB decreases food intake, but this treatment has no effect on food intake in males fed a chow diet (Young, Nance and Gorski, 1979).

In Experiment XI, food intake and body weight were measured in intact male rats fed chow or high fat diet. The effect of diet condition (chow or high fat) on the above measures was also recorded during hormone withdrawal by orchiectomy.

Method.

Animals and housing. Sixty adult male CD-strain rats (Charles River Breeding Laboratories) were housed individually in wire-bottom cages in a room illuminated for 12 hours per day (lights on at 2000 hr). Rats were maintained on Purina rodent chow until the introduction of the experimental diets. Tap water was available throughout the experiment.

Procedure. Two weeks after their arrival in the laboratory rats were assigned to one of two groups (n = 30 each) matched for body weight (Table 10). Groups were high fat diet (HF) and chow diet (C) fed. At the time of group assignment, food pellets were removed from the cages of the HF group and were replaced by glass bowls containing a high fat diet. This diet consisted of 2 parts Purina powdered rat chow and 1 part fat (Crisco). The C group continued to receive Purina rodent chow pellets. The caloric densities of the diets were: HF = 5.46 kcal/g (Young et al., 1978) and C = 3.61 kcal/g (Corbit and Stellar, 1964).

Animals were allowed 1 week to adapt to the diets. Food intake (to the nearest .1 gram; spillage accounted for) and body weight (to the nearest gram) were measured twice weekly after diet adaptation. After two weeks of post-baseline data collection rats were orchiectomized under methoxyflurane anesthesia through a single scrotal incision. Data were collected for 6 weeks post-castration.

Data analysis. Food intake data were converted to k calories. Data were divided into three 3-week blocks (baseline and Weeks 1-2 = pre-gonadectomy; Weeks 3-5 and Weeks 6-8 = post-gonadectomy). A control group to assess the effects of surgery alone was not included in the experiment. However, Gentry and Wade (1976a) found the effects of surgery on food intake and body weight to be limited to the first 3 to 6 post-surgical days. Thus, to minimize the possibility that the decreases reported in the section that follows were due to surgical trauma alone, statistical analyses of data from the first 3-week block following orchiectomy were not included in the results. Comparisons between groups (diets) were analyzed with Student's *t*-test; within group comparisons were made using a *t*-test for paired observations. A *p* value $<.05$ (two-tailed) was considered significant.

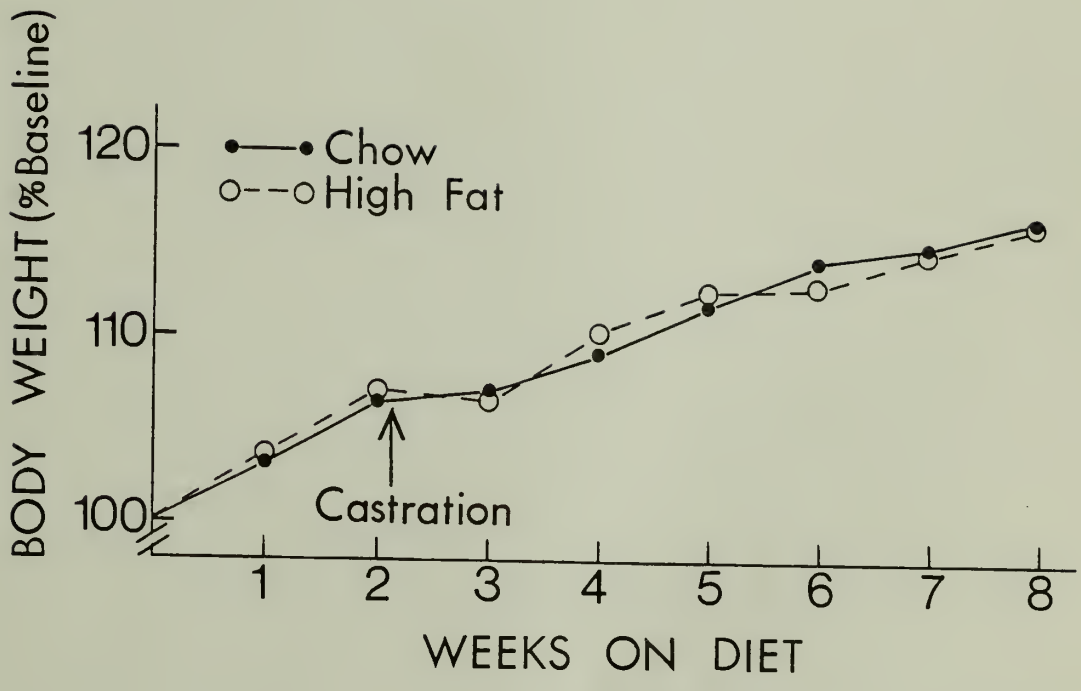



Fig. 15. Effect of castration on body weight in male rats fed chow or high fat diet.

Results.

Body weight (Figure 15, Table 10). The rate of weight gain was not affected by diet. However, castration significantly decreased the rate of weight gain (rate of gain pre-gonadectomy versus rate of weight gain Weeks 6-8; C: $t_{29} = 11.56$, $p<.0001$; HF: $t_{29} = 10.92$, $p<.0001$). During the three weeks prior to castration, the rates of weight gain for the C and HF groups were, respectively, 1.4 $\pm$ .05 g/day and 1.5 $\pm$ .1 g/day. The rates of weight gain for the C and HF groups during Weeks 6-8 were, respectively, .65 $\pm$ .07 g/day and .80 $\pm$ .07 g/day.

Food intake (Figure 16, Table 10). At the start of the experiment food intake did not differ significantly between groups (mean $\pm$ S.E.M.; C: 112.3 $\pm$ 1.6 kcal/day; HF: 107.5 $\pm$ 2.8 kcal/day, $t_{58} = 1.48$, $p>.1$). However, by Week 2 (three weeks on experimental diets) the HF group (mean $\pm$ S.E.M. = 103.0 $\pm$ 2.4 kcal/day) was eating significantly less than was the C group (112.4 $\pm$ 1.5 kcal/day; $t_{58} = 3.34$, $p<.01$). Total daily food intake during the three weeks prior to castration (baseline and Weeks 1 and 2) was significantly less for the HF diet fed rats than for the C fed rats ($t_{58} = 2.44$, $p<.02$). Total food intake remained lower for the HF group than the C group throughout the

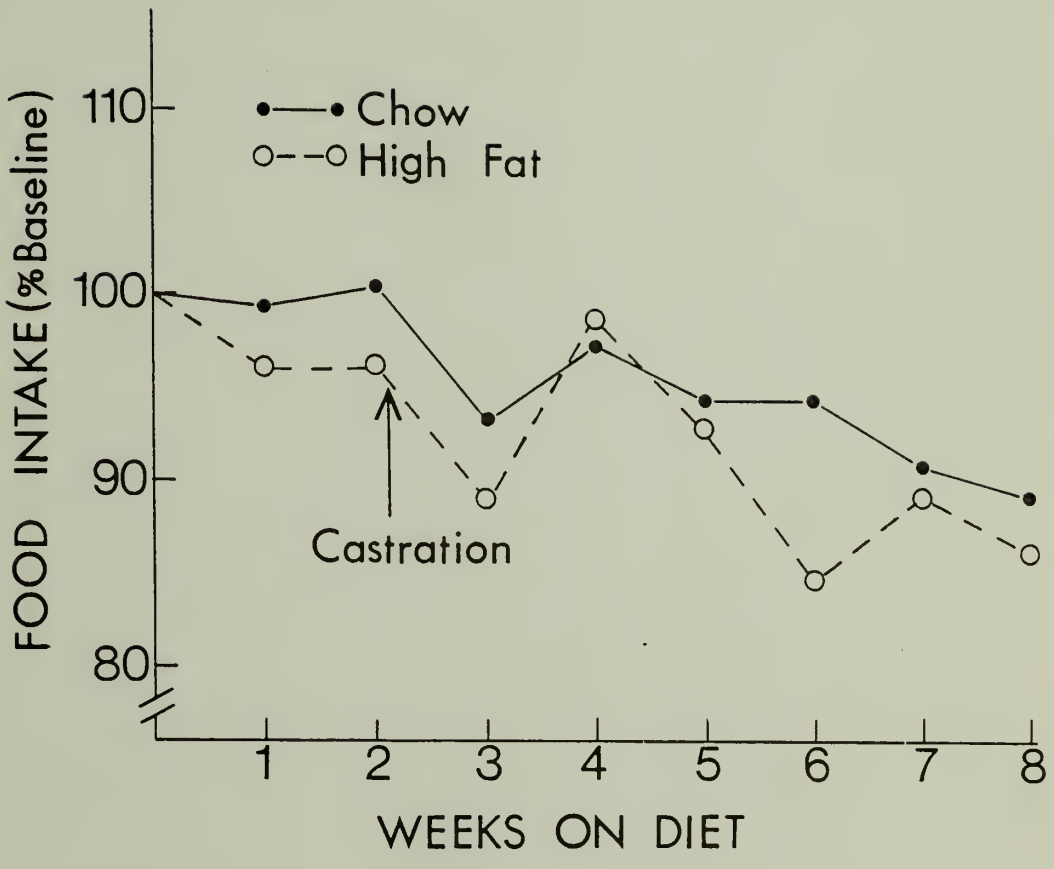



Fig. 16. Effect of castration on caloric intake in male rats fed chow or high fat diet.

TABLE 10

EFFECTS OF CASTRATION ON FOOD INTAKE AND BODY WEIGHT IN RATS FED CHOW OR HIGH FAT DIET
(Mean ± S.E.M.)

| GROUP | BODY WEIGHT (g) (PreGDX) BASELINE | BODY WEIGHT (g) (PostGDX) FINAL | FOOD INTAKE (kcal/day) (PreGDX) WEEKS 0-2 | FOOD INTAKE (kcal/day) (PostGDX) WEEKS 3-5 | FOOD INTAKE (kcal/day) (PostGDX) WEEKS 6-8 |
|---|---|---|---|---|---|
| CHOW | 447.6 ± 5.8 | 523.9 ± 7.0 | 112.3 ± 1.4 | 106.8 ± 1.6 | 100.6 ± 2.9 |
| HIGH FAT | 447.6 ± 6.9 | 524.7 ±11.4 | 105.3 ± 2.5 | 103.0 ± 1.5 | 93.1 ± 2.6 |

post-castration period (Weeks 6-8; $t_{58} = 3.25$, $p<.01$). Castration significantly decreased food intake in both groups (Weeks 6-8; C: $t_{29} = 8.31$, $p<.0001$; HF: $t_{29} = 8.14$, $p<.0001$).

## Experiment XII: Food Intake and Body Weight in GDX Rats Fed Chow or High Fat Diet: Effects of Testosterone Replacement

As discussed in Experiment XI, male or female rats fed a high fat diet are hyperresponsive to the hypophagia-inducing effect of EB. In the present experiment GDX male rats, each receiving a daily injection of either a high or low dose of TP, were fed chow or high fat diet and body weight and food intake were recorded. If high doses of TP decrease food intake (relative to

treatment with a lower dose of TP) via aromatization to estrogens, then male rats fed a high fat diet and treated with high doses of TP should eat less than similarly treated rats fed chow.

Method.

Procedure. Rats were those used in Experiment II. Six weeks after castration, rats within each diet condition were assigned to one of three hormone treatment groups (n = 10 each) matched for body weight and food intake (Table 11). Rats each received a daily injection of either 2 mg TP, .2 mg TP or .1 ml of the injection vehicle alone. Hormones were injected in .1 ml sesame oil. Food intake (including spillage) and body weight were recorded twice weekly. After six weeks of injections, rats were killed with an overdose of Nembutal and anal-nasal lengths were recorded for Lee Index measurements (body weight$^{0.33}$/nasal-anal length; Lee, 1928). Values reported for the Lee Index = Lee Index x 1000 (Table 11).

Data analysis. Body weight data were converted to percent baseline and food intake data were converted to k calories. Data were analyzed using a 2 x 3 factor (Diet x Hormone Treatment) analysis of variance (Winer, 1962). Although no significant Diet x Hormone Treatment interactions were found, significant main

effects of Hormone Treatment ($p<.05$) were followed by Newman-Keuls tests to determine whether both doses of TP were equally effective in influencing the dependent variable at one category of diet (Winer, 1962).

Results.

Body weight (Figure 17, Table 11). At the start of hormone injections, groups did not differ significantly on body weight. After three weeks of hormone treatment, rats eating the HF diet had gained significantly more weight than had the C fed rats, $F$ (1,54) = 4.86, $p<.05$. At Week 3, the groups receiving low doses of TP had gained significantly more weight than those receiving the higher TP doses or the oil vehicle alone, $F$ (2,54) = 24.71, $p<.0001$ ($p<.01$, both diet conditions), and the high dose TP groups weighed more than the oil control groups ($p<.01$, both diet conditions). At the end of the experimental period (Week 6) the two diet groups did not differ significantly on body weight gain, $F$ (1,54) = 3.34, $p>.07$. However, as during the first half of the treatment period, body weight gain was significantly higher in the low dose TP groups than in the high dose TP or oil groups, $F$ (2,54) = 31.45, $p<.00001$ ($p<.01$, all post hoc tests, both diet conditions). Whereas the HF fed 2.0 mg TP group gained significantly more weight than the HF fed, vehicle-treated rats

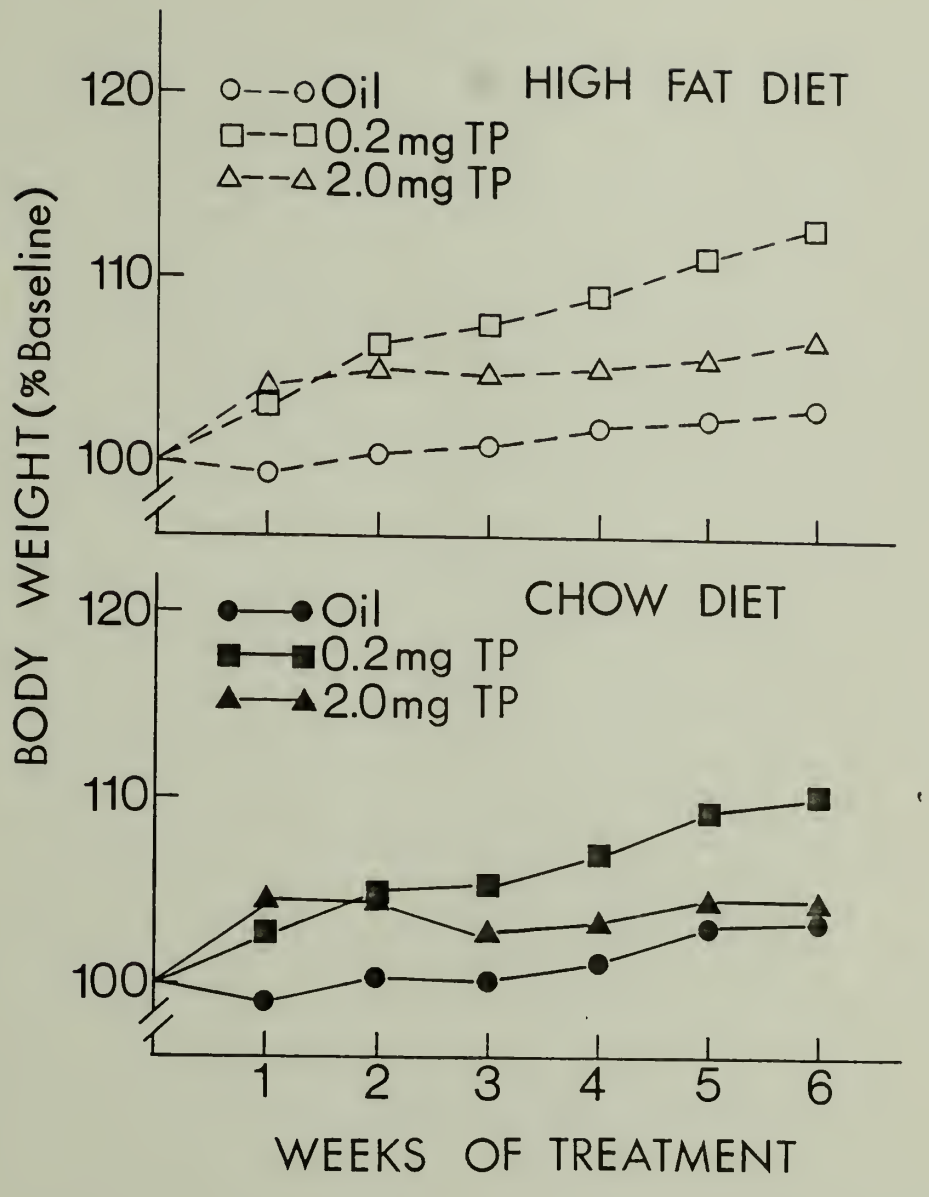



Fig. 17. Effect of long-term treatment with a high or low dose of TP on body weight in GDX rats fed high fat (top) or chow (bottom) diet.

TABLE 11

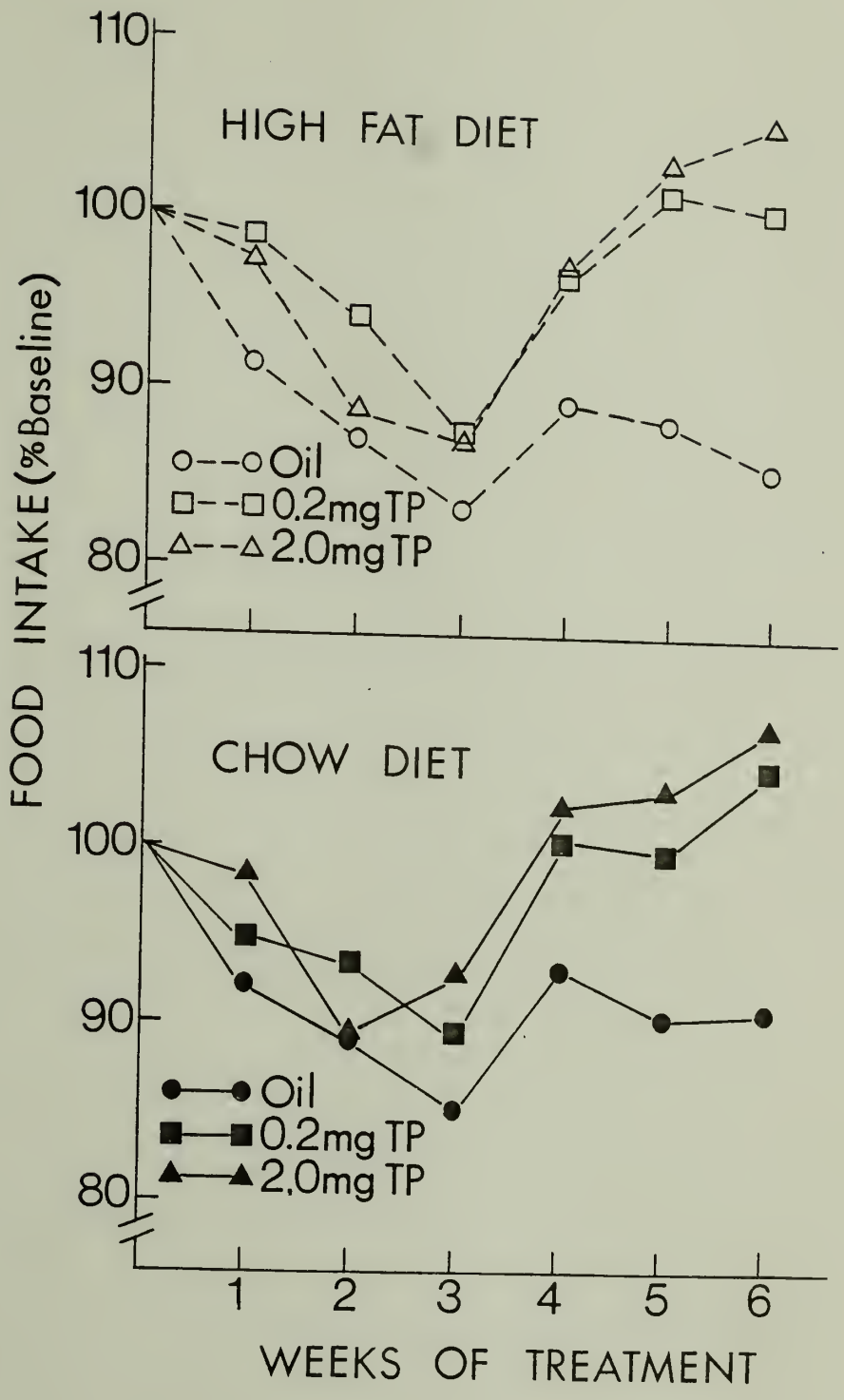
BODY WEIGHT, FOOD INTAKE AND LEE INDEX IN TP-TREATED RATS FED CHOW OR HIGH FAT DIET

| TREATMENT | DIET | BODY WEIGHT (g) | | | FOOD INTAKE (kcal/day) | | | LEE INDEX (x 1000) |
|---|---|---|---|---|---|---|---|---|
| | | BASELINE | WEEK 3 | WEEK 6 | BASELINE | WEEKS 1-3 | WEEKS 4-6 | |
| OIL | CHOW | 523.8 ±10.6 | 532.2 ±12.4 | 543.3 ±13.0 | 101.3 ± 2.5 | 89.9 ±2.5 | 92.4 ±2.9 | 315.7 ± 1.4 |
| 0.2 mg TP | CHOW | 523.0 ±11.8 | 559.3 ±13.4 | 576.2 ±12.9 | 100.5 ± 2.6 | 89.8 ±3.6 | 101.5 ±1.9 | 316.1 ± 1.5 |
| 2.0 mg TP | CHOW | 525.0 ±15.2 | 543.4 ±13.5 | 547.0 ±13.6 | 100.6 ± 3.7 | 93.6 ±2.7 | 104.1 ±2.6 | 312.0 ± 1.1 |
| OIL | HIGH FAT | 528.0 ±24.7 | 538.7 ±26.2 | 545.0 ±28.0 | 92.1 ± 6.2 | 79.8 ±4.3 | 79.8 ±3.2 | 318.3 ± 2.9 |
| 0.2 mg TP | HIGH FAT | 522.0 ±15.7 | 570.6 ±18.2 | 590.6 ±19.8 | 93.0 ± 2.7 | 87.5 ±3.3 | 92.6 ±3.3 | 321.0 ± 1.4 |
| 2.0 mg TP | HIGH FAT | 524.1 ±19.8 | 549.0 ±20.5 | 559.8 ±20.7 | 93.1 ± 4.9 | 84.8 ±4.2 | 94.2 ±4.2 | 319.4 ± 1.9 |

($p<.05$), C fed rats receiving the high dose of TP did not differ significantly from the vehicle-treated control group.

Food intake (Figure 18, Table 11). At the beginning of the injection period, rats fed the HF diet were eating significantly less than were C fed rats, $F$ (1,54) = 5.95, $p<.02$. No other differences across groups were found on baseline intake measures. During the first three weeks of hormone treatment, rats fed the C diet consumed significantly more calories than did the rats eating the HF diet, $F$ (1,54) = 6.11, $p<.02$. No significant Hormone or Hormone x Diet interaction effects were found at this time ($F$ (2,54) = .936, $p>.39$; $F$ (2,54) = .709, $p>.49$, respectively). During the final three weeks of the experiment, food intake of HF fed animals was still significantly lower than that of C fed rats, $F$ (1,54) = 17.06, $p<.0001$. However, during this last half of the experimental period (unlike the first half), a significant Hormone Treatment effect was found, $F$ (2,54) = 10.19, $p<.0001$. Both doses of TP significantly increased food intake compared to oil controls ($p<.01$, C fed; $p<.05$, HF fed). Caloric intakes of the 2.0 mg TP groups did not differ significantly from those of the low dose TP groups.

Fig. 18. Effect of long-term treatment with a high or low dose of TP on caloric intake of GDX rats fed high fat (top) or chow (bottom) diet.


110
100
90
80
HIGH FAT DIET
Oil
0.2mg TP
2.0mg TP
110
100
90
80
CHOW DIET
Oil
0.2mg TP
2.0mg TP
FOOD INTAKE (%Baseline)
1
2
3
4
5
6
WEEKS OF TREATMENT

Lee index. Lee index measurements are given in Table 11. Analysis of variance revealed a significant main effect of Diet, $F$ (1,54) = 11.18, $p<.001$, whereas no effect of Hormone Treatment ($F$ (2,54) = 1.22, $p>.29$) and no Diet x Hormone Treatment interaction was found ($F$ (2,54) = .88, $p>.4$). Thus, although body weights of HF and C fed rats did not differ at the end of the experiment, rats fed the HF diet were fatter than C fed rats.

## Discussion

In male rats, the rates of weight gain following castration were not affected by diet. In agreement with earlier findings, orchiectomy decreased the rate of body weight gain (in chow fed rats; Gentry and Wade, 1976a). Whereas food intakes did not differ between groups during the first week on the experimental diets (baseline), total food intake for the HF group fell significantly below that of the C group during the remainder of the experiment. A decrease in caloric intake compared to initial intake has been reported previously for female rats fed a HF diet (Corbit and Stellar, 1964). That the decrease in food intake in HF diet fed males (compared to C fed rats) was a response to the HF diet and not due to castration is

supported by the fact that males consuming the HF diet still maintained their intakes at levels lower than those of C fed males when replacement hormones were given. In the present experiment, castration significantly decreased food intake in rats in both diet groups. This is in contrast to the findings of Young, Nance and Gorski (1979) who reported no effect of castration on food intake in HF fed male rats. In that study male rats fed a HF diet consumed a significantly greater number of calories than did C fed rats.

It was predicted that food intake of HF diet fed rats receiving the high dose of TP would be decreased relative to that of rats receiving similar hormone treatment and fed a C diet. The above decrease would be expected to occur after about two weeks of hormone treatment since it is at this time that the estrogenic effects of high doses of TP on body weight gain are observed. In Experiment XII, no evidence was found to suggest that HF fed rats treated with high doses of TP were hyperresponsive to the effects of estrogenic metabolites of TP compared to similarly treated males fed the C diet. That is, no Diet x Hormone Treatment interaction was found during any period of hormone administration. In addition, individual comparisons (*t*-tests; data not shown) on the percent change in food

intake from baseline levels at each week of hormone treatment for the HF and C fed rats receiving injections of 2.0 mg TP revealed no significant differences between groups at any time. The present results do, however, replicate previous findings on the effects of .2 mg and 2.0 mg doses of TP on food intake and body weight gain in GDX, C fed rats (Experiment I; Gentry and Wade, 1976a).

It is not known why high doses of TP failed to suppress food intake in HF fed males compared to C fed, 2.0 mg TP-treated rats. Chow fed, GDX male rats are relatively insensitive to the influences of EB on food intake compared to female rats (Gentry and Wade, 1976b; Young, Nance and Gorski, 1979). Young, Nance and Gorski (1979), found that one injection of EB suppressed food intake in HF fed GDX males, whereas this treatment did not reduce food intake in C fed animals. In that experiment the rats eating the HF diet ate significantly more prior to hormone treatment (103.9 kcal/day) than did the C fed animals (86.9 kcal/day). It is possible that this effect of estrogen is only manifest when food intake of HF fed rats is initially higher than that of C fed rats.

It is interesting to note that the HF diet fed rats ate significantly fewer calories throughout the

experiment compared to C fed rats, yet both groups gained weight at similar rates. This finding may possibly be explained by examining the metabolic costs for storage of the different dietary components contained in each diet. Since rats in both diet groups were gaining weight, it may be assumed that the amounts of food consumed exceeded the animals' immediate caloric requirements and excess fuels were stored for later use (see Friedman and Stricker, 1976 for review). The C diet contained approximately 22.8% protein, 4.5% fat and 56% carbohydrate (or about .9 kcal/g and 2.3 kcal/g C diet, respectively). The percentages of protein, fat and carbohydrate for the HF diet were, respectively, 15.3% (.6 kcal/g HF diet), 36.3% (3.3 kcal/g) and 37.3% (1.5 kcal/g). Flatt (1978) has estimated that the dissipation of energy through storage (including allowance for recycling) is 24% for protein, 7% for fat, and 4% and 28% for carbohydrate when it is stored, respectively, as glycogen or fat. From the estimations above, it is suggested that less energy was required to store the HF diet than the C diet, and thus rats eating the HF diet could consume fewer calories than the C fed rats and still maintain a similar level of body weight.

It is not my intention to imply that the above analysis of metabolic energy expenditure is by any

means complete. However, that rats fed the HF diets stored more calories as fat (and fat provided the majority of calories in the diet and required relatively little energy for direct storage in adipose tissues) is suggested by the significantly higher Lee index ratings of the HF group compared to the C groups. In fairness, it should be stated that there is disagreement in the literature as to whether the Lee index provides a good measurement of obesity (increased body fat; e.g., Bernardis and Patterson, 1968; but c.f. Stephens, 1980). Earl Simson (manuscript in preparation, 1981) has data which suggest that the Lee index correlates well with dietary-induced increases in body fat, but not with hormone-dependent (e.g., OVX-induced) obesity. In Experiment VII, nasal-ano lengths were recorded for Lee index measurements in addition to determination of body fat content by direct carcass analysis. Indeed, in that experiment, the correlation between percent carcass lipid and the Lee index rating was only .42, although the mean Lee index rating for each hormone treatment group showed the same direction of change as the mean percent lipid for each group. (The mean ($\pm$S.E.M.) Lee index and the mean ($\pm$S.E.M.) percent wet carcass lipid for the treatment groups were, respectively: Oil - 317.5 $\pm$ 2.7, 21.9 $\pm$ .8%; 2.0 mg DHTP - 319.8 $\pm$ 2.4,

22.2 ± .8%; 0.2 mg DHTP - 320.7 ± 3.0, 22.1 ± .8%).

In summary, no evidence was found to suggest that male rats fed a high fat diet are hyperresponsive to the anorexic effects of estrogenic metabolites of testosterone. High fat and chow fed GDX rats responded similarly to androgen replacement therapy in terms of body weight and food intake, although the daily caloric intake of rats fed the high fat diet was consistently lower than that of rats eating standard laboratory chow.

# CHAPTER VIII

## GENERAL DISCUSSION

### Major Findings

Castration of male rats significantly decreases food intake and body weight gain (Experiment XI). When castrated male rats are given long-term treatment with .2 mg TP, food intake and body weight gain increase compared to GDX rats receiving the injection vehicle alone (Experiments I, V, VI, XII). This dose of TP (200 µg/day) has been shown to restore circulating plasma testosterone titers to pre-castration levels in adult orchiectomized male rats (Patsch, Kim, Wiest and Schonfeld, 1980). The increased food intake of GDX rats given daily injections of this relatively low dose of TP is, in general, due to enhanced consumption of all dietary components (Experiments V and VI).

Postmortem carcass analyses revealed that the increased body weight of the .2 mg TP-treated group was associated with elevated carcass protein content compared to the GDX, oil-treated group (Experiment VI). Low doses of TP not only increase carcass protein (on a per gram basis), carcass lipid is also significantly increased by this treatment compared to oil or high

dose TP treatment. This increase in fat is clearly not due to changes in LPL activity in epididymal adipose tissue (Experiment II). Some of the increased fat of males treated with low doses of TP may be due to incorporation of lipids into muscle during TP-stimulated muscle growth. However, there is virtually no information on other mechanisms which increase adiposity in male rats. Little information is available on the influence of testosterone on enzymes involved in triglyceride storage and clearance in rats. With the exception of one study which found a sex difference in hepatic apoproteins that is controlled by testosterone (Patsch et al., 1980), no studies have systematically investigated the effects of castration and androgen replacement on triglyceride metabolism in male rats.

When GDX male rats are treated with higher doses of TP (1 or 2 mg/day) body weight gain and food intake initially increase such that they generally do not differ significantly from those of GDX rats given daily injections of .2 mg TP (Experiments I, V, VI, XII). However, after approximately one week, body weight gain and food intake of rats receiving the higher doses of TP begin to decrease relative to those of rats given the lower concentrations of TP. After

one to two months of treatment with high doses of TP, body weight has fallen such that it is significantly lower than that of rats treated with .2 mg doses of TP (Experiments I, V, VI, XII); and it is similar to (and not significantly different from) weight of oil-treated controls (Experiments I, V, IX, XII). On the other hand, rats given high doses of TP show a transient decrease in food intake (relative to .2 mg TP-treated rats), but with extended treatment food intake increases such that it does not differ significantly from that of rats given lower doses of TP (Experiments I, VI, XII). Note, however, that when rats were allowed to self-select from two isocaloric diets differing in protein and carbohydrate contents (Experiment V) total caloric intake was significantly higher in .2 mg TP-treated rats than in rats given 2.0 mg doses of TP. Food intake of rats given long-term treatment with high doses of TP is higher than that of oil-treated rats (this increase was significant in Experiments V, VI and XII, but not in Experiments I or IX).

Treatment with the 2.0 mg dose of TP causes a selective increase in protein intake. Protein intake of GDX rats treated with high doses of TP does not differ from that of rats given .2 mg TP (Experiments V and VI). These data support the contention that the

increased food intake of chow fed rats given high doses of TP actually reflects an attempt to acquire a greater quantity of a specific macronutrient, protein. Indeed, total caloric intake of self-selecting GDX rats given high doses of TP was significantly less than that of self-selecting rats given .2 mg doses of TP. The elevated protein intake of rats treated with 2.0 mg doses of TP was associated with increased carcass protein content (as it was in rats given lower doses of TP). The decreased weight of rats treated with the high dose of TP is due to a reduction in body fat content (Experiment VI).

Data have been presented which strongly suggest that both the decreased body weight and adiposity of GDX male rats given extended treatment with high doses of TP are due to estrogenic metabolites of TP (Experiments I and II). First, concurrent treatment with ATD, which blocks the aromatization of androgens to estrogens, prevents the weight-reducing effects of high doses of TP. Second, long-term treatment with high (1.0 mg/day), but not low (.2 mg/day), doses of TP depletes cytoplasmic estrogen receptors in male rat adipose tissues, possibly by causing translocation of the receptor-hormone complexes into cell nuclei. Gray, Dudley and Wade (1981) have demonstrated an

accumulation of radioactivity in nuclei of adipose tissue cells following an injection of [$^{3}$H]estradiol in GDX male or female rats. The estrogen specificity of the receptor depletion in the high dose TP-treated males is demonstrated by its absence in rats given simultaneous treatment with 1.0 mg doses of TP and the aromatization inhibitor, ATD. Third, repeated daily injections of 1.0 mg TP (but not .2 mg TP) reduce LPL activity (by approximately 60%) in epididymal adipose tissues. A similar (75%) reduction in adipose tissue LPL activity is observed following EB treatment in GDX male rats (Gray and Wade, 1980). Again, the effects of high doses of TP on epididymal adipose tissue LPL activity are blocked by concurrent treatment with ATD. Finally, injections of the non-aromatizable androgen, DHTP, increase (but do not decrease), food intake and body weight gain, even when given in high doses (Experiment VII). These data support the hypothesis that aromatized metabolites of testosterone may, in part, reduce body weight and adiposity by direct actions on adipose tissue metabolism, including LPL activity. Thus, estrogenic metabolites of testosterone affect body weight and adiposity by a mechanism similar to that by which estrogens affect changes in these variables in female

rats. In addition, estrogenic metabolites of TP may also act at central (hypothalamic) sites to decrease food intake in male rats (Experiments III and IV), just as hypothalamic implants of EB reduce food intake in female rats.

Although data above provide support for the hypothesis that reductions in body weight and adiposity that follow treatment with high doses of testosterone are due to aromatized metabolites of the androgen, they do not explain why a lag period exists between injections of the hormone and the observation of decreases in these measures. That is, in rats given daily injections of 2.0 mg doses of TP, body weight increases for approximately one week, and it is only after this time that the attenuation in weight gain is observed. This question cannot be answered directly since although it is known that human adipose tissues contain the enzymes necessary for aromatization of androgens to estrogens (Kley et al., 1980; Nimrod and Ryan, 1975; Perel and Killinger, 1979), investigators have not yet attempted to measure aromatase activity in adipose tissues in rats. The role played by gonadal steroids in regulation of aromatase activity in human adipose tissue is not known.

Nonetheless, one explanation for the seven to ten

day lag period may be that testosterone titers must be elevated above normal (unmated) circulating levels for an extended period of time in order to induce aromatase activity that is sufficient to override the weight-increasing effects of testosterone. In the central nervous system, castration decreases and androgens increase aromatase activity in rats (Kobayashi and Reed, 1977). The temporal requirements for induction of aromatase activity by testosterone have not been systematically studied. However, there is evidence that under certain circumstances in human males, prolonged administration of testosterone is necessary to induce aromatase activity in some systems. For example, acute testosterone treatment does not affect pituitary responsiveness to gonadotropin-releasing hormone (GnRH). However, after two to four weeks of testosterone treatment, pituitary responsiveness to GnRH decreases sharply, just as it does following short-term estradiol treatment (Bardin, 1980). The apparently long lag period for induction of a specific protein by testosterone is not unprecedented. The hepatic protein, $\alpha_{2u}$-globulin, is found in adult male rats, but is absent in adult females. Castration decreases $\alpha_{2u}$-globulin in males, and testosterone treatment of GDX male or female rats induces $\alpha_{2u}$-globulin to levels near those of intact

male rats after eight days (Bardin and Catterall, 1981).

As discussed more fully in Chapter V, copulation-induced reductions in body weight may be mediated by mechanisms other than those mediating the decreases in weight that follow prolonged treatment with high doses of TP in castrated rats. Body weight gain was decreased in rats allowed to copulate, but this decrease was not associated with changes in caloric intake or in the intake of specific nutrients. Furthermore, LPL activity in epididymal fat depots was not altered in mated male rats (Experiment VIII). Several researchers have reported that copulation increases plasma testosterone and luteinizing hormone (LH) levels (Graham and Desjardins, 1980; Kamel and Frankel, 1978; 1979; Kamel et al., 1975). In contrast, Balin and Schwartz (1976) failed to find increased plasma LH in mated male rats (even when serum sampling took place every 15 minutes during the hour following completion of mating tests), yet rats in that study had significantly higher seminal vesicle weights than unmated males (testosterone was not measured). The results of Experiment VIII suggest that the reduced body weights of mated male rats are not mediated solely by testicular testosterone. Further work is needed to ascertain whether the changes in adiposity and body weight of males given unlimited access

to receptive females are mediated by sustained increased levels of endogenous testosterone. Unfortunately, it is not known if repeated injections of 2.0 mg TP/day produce plasma testosterone titers similar to the circulating levels of testosterone in intact male rats housed with females.

## Summary and Conclusions

Testicular steroid hormones exert marked effects on food intake, body weight and carcass composition in male rats. Some information exists on mechanisms by which androgens affect the latter two variables, but the evidence suggesting how these hormones influence food intake is scarce. Food intake and body weight gain do not always change in parallel as would be expected if weight gain directly reflected food intake (e.g., Experiment I). Provided below is a scheme which summarizes how androgens may influence the above measures in male rats (some of the influences of testosterone on eating and body weight are summarized in Figure 19). It should be cautioned that this view is admittedly (and necessarily) derived from a limited group of data and is incomplete; but as stated by George Wade, whose work has contributed greatly to an understanding of how gonadal hormones affect food intake,

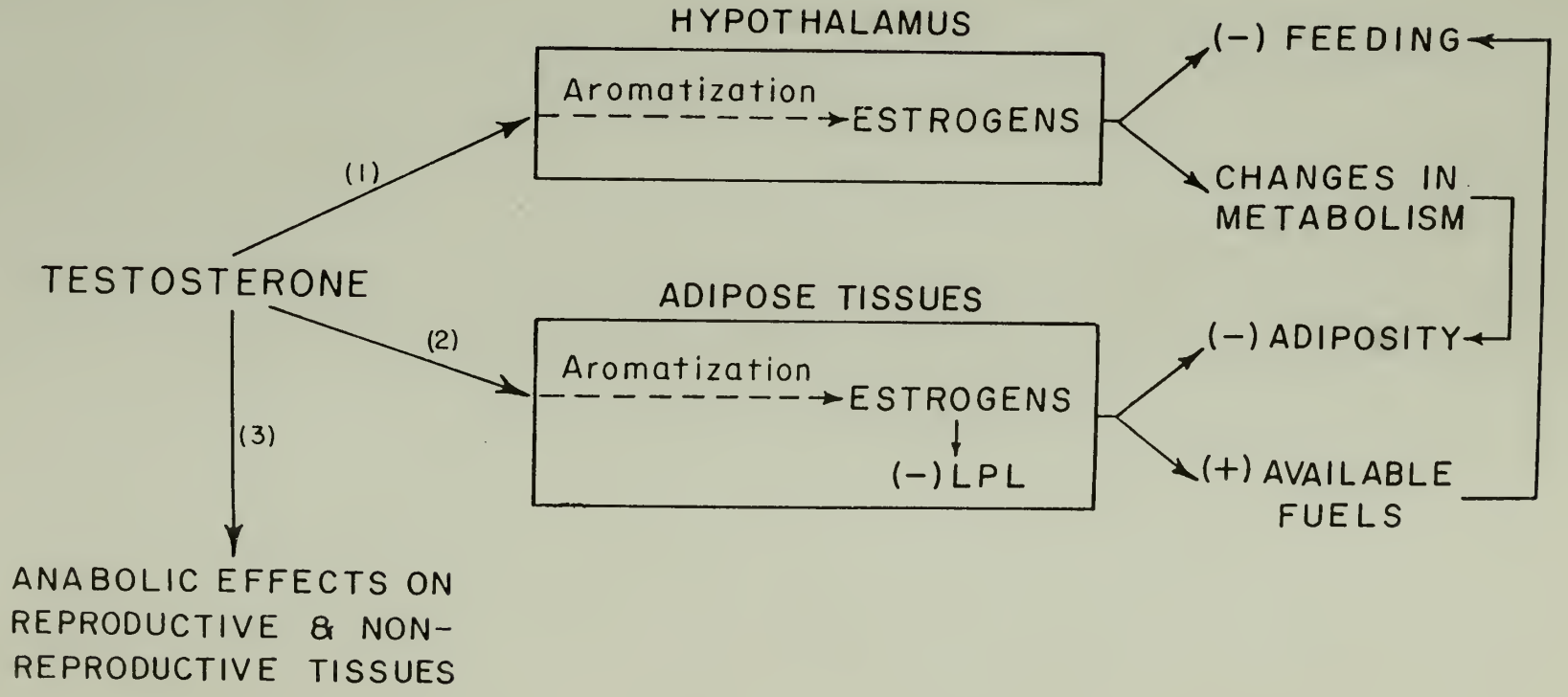



Fig. 19. Schematic diagram illustrating some of the ways testosterone may act to influence food intake, body weight and carcass composition in adult rats.

body weight and adiposity in rats: "In the absence of data, one is free to speculate."

Testicular hormones may act at several sites. Two target sites are the hypothalamus and pituitary which contain steroid-specific, saturable cytoplasmic estrogen (Eisenfeld, 1970), progestin (Kato and Onouchi, 1977; Maclusky and McEwen, 1978) and androgen receptors (Barley et al., 1975; Naess, Attramadal and Aakvaag, 1975). The hormone-receptor complexes are concentrated in cell nuclei and alter both RNA and protein synthesis (Blaustein and Wade, 1978; Lieberburg and McEwen, 1977; Zigmond and McEwen, 1970).

Androgens may exert several central effects, primarily via the hypothalamus. That testicular hormones induce aromatase activity in several hypothalamic sites is shown by the finding that castration reduces aromatase activity in specific hypothalamic nuclei to levels observed in female rats (Kobayashi and Reed, 1977). Thus, androgens, primarily testosterone, and its estrogenic metabolites, may act in several of the following ways. First, the hormones might exert direct effects on eating behavior. As has been demonstrated, VMH implants of TP or EB decrease food intake in male rats (Beatty et al., 1974; Experiments III and IV). However, it is not known if the change in food intake

is by direct neural control of ingestive behaviors. Second, hormones may act in the hypothalamus to influence metabolism via the autonomic nervous system. For example, the hypothalamus has been shown to influence the endocrine pancreas via autonomic nerves (Woods and Porte, 1974). Testosterone might influence the hypothalamus to increase insulin secretion, thus increasing body weight. However, this cannot be the only mechanism by which androgens act, since the anabolic effects of testosterone are unaltered in castrated alloxan-diabetic male rats (Kochakian, 1975). Carcass fat was not measured in that study, but testosterone increases adiposity and nitrogen retention in GDX, diabetic male dogs (Kochakian, 1975). Third, testosterone and estradiol could exert indirect effects on metabolism by influencing hypothalamic releasing hormones. For example, estradiol increases plasma prolactin in male rats (Tresguerres, Esquifino, Perez Mendez and Lopez-Calderon, 1981), and prolactin decreases adipose tissue LPL (Zinder, Hamosh, Fleck and Scow, 1974), as does estradiol. Furthermore, prolactin acts synergistically to potentiate the effects of testosterone in muscle and in male reproductive tissues (Bardin, 1980; Mainwaring, 1978).

Gonadal hormones may also act directly on the pituitary. Testosterone, for example, may act at the pituitary (and/or hypothalamus) to influence secretion of growth hormone. Growth hormone has anabolic actions similar to those of testosterone, and it has also been shown to stimulate food intake (Kochakian, 1975; Martin, 1976). However, that the effects of testosterone are not mediated by growth hormone is evidenced by the fact that testosterone influences these measures in hypophysectomized male rats (Kochakian, 1975). In addition, the concurrent administration of testosterone and growth hormone produces an additive effect on nitrogen retention and body weight gain (Kochakian, 1975).

Thus, in addition to direct actions on behaviors, testicular hormones may act in the central nervous system and pituitary to bring about metabolic changes which, in turn, indirectly influence food intake, body weight and carcass composition. Testosterone may also affect secretion of certain pituitary hormones which potentiate the effects of the steroid.

Testicular estrogens or estrogenic metabolites of testosterone formed locally, may also act directly on adipose tissues via cytoplasmic estrogen receptors (Wade and Gray, 1978) to reduce LPL activity. The reduction in enzyme activity may decrease body weight and

adiposity and result in increased availability of triglycerides as utilizable fuels for use in other tissues. Altered fuel levels could consequently change food intake, but as discussed earlier, information concerning hormonal effects on triglyceride metabolism in male rats is virtually nonexistent.

Finally, androgens have growth-promoting effects on many peripheral tissues containing androgen-binding proteins. Testosterone has anabolic effects on tissues such as skeletal muscle and kidney (Bardin et al., 1978; Kochakian, 1975; Scow, 1952). Anabolic actions in liver appear to be mediated by the testosterone metabolite, androstenedione (Gustafsson, Pousette, Stenberg and Wrange, 1975; Mainwaring, 1978). Testosterone and DHT stimulate growth in male reproductive tissues.

In summary, androgens may act at multiple sites to alter food intake, body weight and carcass composition in male rats. The work presented in this dissertation investigated some of the sites at which androgens act and some mechanisms by which they act to influence these variables.

"Oyf itlekhn terets ken men gefinen a naye kashye."
(There is a new question to every answer.)

Old Yiddish Proverb

## FOOTNOTES

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